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Welcome Remarks

Dear conference participant,

On behalf of the conference committee, we cordially welcome you at the

2nd Annual Meeting of NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research
26th – 28th November 2009 at Henry-Ford Building, FU Berlin.

This conference convenes outstanding scientists in the field of medical genome research. The event offers the exceptional opportunity of information about the latest developments, presentation of scientific results, and discussion and interaction with most competent researchers in a highly dynamic atmosphere.

The successful start of the future-oriented new Program of Medical Genome Research, launched by the German Federal Ministry of Education and Research (BMBF), was celebrated with more than 450 participants at the 1st Annual Meeting of NGFN-Plus and NGFN-Transfer 2008 at the Helmholtz Zentrum München.

We are delighted about the even increasing interest in the meeting 2009, documented by more than 650 registered participants, as well as an overwhelming number of abstract contributions for oral and poster presentations. We are also happy to welcome internationally renowned keynote speakers and to convene the experts in German medical genome research in the friendly atmosphere of the Henry-Ford Building at FU Berlin. For their generous support to host this conference in Berlin, we thank Prof. Hans Lehrach and Prof. Bernhard Herrmann, Max Planck Institute for Molecular Genetics, and Prof. Volker A. Erdmann, Freie Universität Berlin.

The enormous diversity of the NGFN is shown in six symposia entitled *Genomics of Common Disease, Genomics of Sporadic Cancer, Animal, Cellular & Tissue Models, Systems Biology, New Technologies,* and *Transfer from Genomics to Application*. Internationally renowned keynote speakers will open the sessions with overview lectures. Scientists of the NGFN will then present their latest results.

The conference starts with two workshops that focus on most relevant topics: *Next-Generation Sequencing* and *Epigenetic Regulation.* The presentation of novel results by employing powerful cutting-edge technology will demonstrate impressive results in medical genome research.

To all six symposia the conference offers poster sessions. In order to motivate young scientists, to strengthen the importance of the poster presentation, and in memory of the late Prof. Dr. Annemarie Poustka, three posters will receive the “Annemarie Poustka Poster Award for Medical Genome Research 2009” sponsored by Roche Diagnostics. Annemarie Poustka made outstanding achievements in the field of Genome Research and was a visionary scientist for the NGFN.

Company satellite lunch sessions round the program off and an industrial exposition offers comprehensive information on latest technology development in supply relevant for researchers of the network.

To all members of NGFN-Plus and NGFN-Transfer, this is a great opportunity for meeting each others within the BMBF Program for Medical Genome Research, to see new and "old" collaborators and to reinforce existing co-operations. We are pleased to welcome many members of NGFN-1 and NGFN-2, all those scientists that are interested in the program as well as all further visitors of our conference to take actively part in the scientific discussion and exchange.

Last but not least, don't miss the highlight Evening Lecture, followed by a get-together with the scientific community in a relaxed ambience with good wine, tasty finger food and nice music!

Munich and Heidelberg, Nov. 18, 2009

Prof. Martin Hrabĕ de Angelis

Prof. Hugo A. Katus

(As Spokespersons for the Project Committee of NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research)

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Helmholtz Zentrum München

PD Dr. Stefan Wiemann
DKFZ Heidelberg

Prof. Wolfgang Wurst
Helmholtz Zentrum München

Program-at-a-glance

Thursday, November 26th

1:00 – 4:00 pm	**Workshop: Next Generation Sequencing**
4:00 – 4:30 pm	***Coffee Break***
4:30 – 6:30 pm	**Workshop: Epigenetic Regulation**
7:00 pm	***Supper***

Friday, November 27th

8:30 - 10.30 am	**Symposium I – Genomics of Common Disease** Heribert Schunkert (Keynote) - Christian Gieger - Anke Hinney – Sven Cichon – Andre Franke – Margret Hoehe
10:30 - 11.00 am	**Coffee Break**
11.00 – 11:30 am	**Welcome:** **Hans Lehrach,** Max Planck Institute for Molecular Genetics, Berlin, Germany **Helge Braun (MdB), Parlamentarischer Staatssekretär** im Bundesministerium für Bildung und Forschung **Martin Hrabé de Angelis**, Helmholz Zentrum München, Germany, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research
11:30 – 1:00 pm	**Symposium II – Genomics of Sporadic Cancer** Ivo Gut (Keynote) - Vegi M. Naidu - Özgür Sahin - Johannes Schulte
1:00 – 3:00 pm	***Lunch Break and Poster Session I***
1:00 – 3:00 pm	**Company Satellite Lunch Sessions** Illumina Ltd - Roche Diagnostics GmbH– Applied Biosystems
3:00 – 4:45 pm	**Symposium III – Animal, Cellular and Tissue Models** Keynote - Bernhard G. Herrmann - Till Acker – Deike Hesse - Guido Krebiehl
4:45 – 5:15 pm	**Coffee Break**
5:15 – 7:00 pm	**Symposium IV – Systems BIology** Hans Westerhoff (Keynote) - Hans Lehrach - Bodo Lange - Björn Schwanhäusser - Jörn Walter
7:00 – 8:00 pm	**Evening Lecture:** Jens Reich
8:00 – 10:00 pm	***Get-Together (Wine, fingerfood, music)***

Saturday, November 28th

9:00 - 10.45 am	**Symposium V – New Technologies** Erich Wanker (Keynote I) - Jan Korbel (Keynote II) - Hans-Jörg Warnatz - Andreas Schlicker - Mihail Sarov
10:45 – 12:45 pm	***Lunch Break and Poster Session II***
10:45 – 12:45 pm	**Company Satellite Lunch Sessions:** Febit Biomed GmbH – Affimetrix Europe – Fluidigm Europe B.V.
12:45 – 2:45 pm	**Symposium VI: Transfer from Genomics to Application** Jörg Rademann (Keynote) – Nikolaus Machuy – Andreas Keller – Ruprecht Kuner – Ulrike Korf - Patrick Linsel-Nitschke
2:45 – 3:00 pm	**Ceremony: "Anemarie Poustka Poster Award of Medical Genome Research 2009" sponsored by Roche Diagnostics GmbH** **Concluding Remarks:** Hugo A. Katus, Universitätsklinik Heidelberg, Speaker Project Committee of NGFN-Plus/NGFN-Transfer in the Program of Medical Genome Research

2nd Annual Meeting NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research

Henry-Ford Building, FU Berlin

Workshop
Next Generation Sequencing
November 26th 2009

Scientific Organization:		**Ralf Sudbrak**
1:00 pm	Introduction	**Ralf Sudbrak**
Session 1	Talks 15 min plus 5 min discussion	**Chair: Peter Nürnberg, Köln**
1:10 pm	The Neandertal Genome Project **Adrian Briggs, Leipzig**	
1.30 pm	The 1000 Genome Project **Hans Lehrach, Berlin**	
1.50 pm	The International Cancer Genome Project **Peter Lichter, Heidelberg**	
2.10 pm	Discussion	
Session 2	Talks 15 min plus 5 min discussion	**Chair : Matthias Platzer, Jena**
2.20 pm	Detection of tumor-specific somatic mutations by transcriptome sequencing of a cytogenetically normal acute myeloid leukaemia **Sebastian Eck, Munich**	
2.40 pm	From genetic etiology to transcriptome patterns - Ultrafast sequencing as a tool to understand human chronic inflammatory diseases **Philip Rosenstiel, Kiel**	
3.00 pm	High Throughput Sequencing of Coding Regions in Alzheimer's Disease using Next Generation Technologies **Lars Bertram, Berlin**	
Session 3	**Outlook**	**Chair : Thomas Meitinger, München**
3.20 pm	Next Generation Sequencing: An Outlook **Gertjan van Ommen, Leiden** (Talk as an opener for the following discussion)	
3.40 pm	Discussion	
4.00 pm	End of Workshop	
4.00 pm – 4.30 pm	**Coffee Break**	

2nd Annual Meeting NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research

Henry-Ford Building, FU Berlin

Workshop
Epigenetic Regulation
November 26th 2009

Scientific Organization: **Peter Lichter**

Sessions		Talks 15 min plus 5 min discussion
Chair: Peter Lichter, Heidelberg		
4:30 pm	Epigenomics in Chronic Lymphocytic Leukemia **Christoph Plass, Heidelberg**	
4.50 pm	Cis-acting factors determine DNA methylation states in health and disease **Michael Rehli, Regensburg**	
5.10 pm	Epigenetic restriction during cellular differentiation **Dirk Schübeler, Basel**	
5.30 pm	The clinical relevance of epigenetic alterations in leukemia **Carsten Müller-Tidow, Münster**	
5.50 pm	Characterization of age- and environment-dependent DNA methylation changes **Frank Lyko, Heidelberg**	
6.10 pm	Joint Discussion	All
6.30 pm	End of Workshop	
7.00 pm	**Supper**	

Program

Thursday, November 26th, 2009

1:00 – 4:00 pm	**Workshop: Next Generation Sequencing** (Org. Hans Lehrach, Ralf Sudbrak)
4:00 – 4:30 pm	***Coffee Break***
4:30 – 6:30 pm	**Workshop: Epigenetic Regulation** (Org. Peter Lichter)
7:00 pm	***Supper***

Friday, November 27th, 2009

Symposium I: Genomics of Common Disease

8:30 – 9:15 am	**Keynote: Heribert Schunkert**, University Clinics Lübeck, Germany *Genetics of myocardial infarction*
9:15 – 9:30 am	**Christian Gieger,** Helmholtz Zentrum München, Germany *The role of genetically determined metabotypes in the genetics of complex traits and polygenic disorders.*
9:30 – 9:45 am	**Anke Hinney**, University Duisburg-Essen, Germany *Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early onset extreme obesity in French and German study groups*
9:45 – 10:00 am	**Sven Cichon**, Institute of Human Genetics, University of Bonn, Gemany *Genome-wide survey implicates CNVs in early-onset bipolar disorder*
10:00 – 10:15 am	**Andre Franke**, Christian-Albrechts-University zu Kiel, Germany *Whole-genome, -exome, and targeted resequencing approaches to identify the remaining genetic heritability in Crohn's disease*
10:15 – 10:30 am	**Margret Hoehe**, Max Planck Institute for Molecular Genetics, Berlin, Germany *MHC Haplotype Sequencing: An Integrated Approach to Common Disease*
10:30 – 11:00 am	***Coffee Break***

Welcome

11:00 – 11:30 am	**Hans Lehrach,** Max Planck Institute for Molecular Genetics, Berlin, Germany **Helge Braun (MdB),** Parlamentarischer Staatssekretär im Bundesministerium für Bildung und Forschung **Martin Hrabé de Angelis**, Helmholtz Zentrum München, Speaker Project Committee of NGFN-Plus/NGFN-Transfer in the Program of Medical Genome Research

Symposium II: Genomics of Sporadic Cancer

11:30 – 12:15 pm	**Keynote: Ivo Gut**, CEA/ Institut de Génomique-Centre National de Génotypage, Evry, France *From genome-wide association studies to whole-genome sequencing in cancer*
12:15 – 12:30 pm	**Vegi M. Naidu**, LMU, Munich, Germany *AML1-ETO collaborates with the HOX Gene Co-Factor, MEIS1 in inducing acute leukemia in the mouse bone marrow transplantation model*
12:30 – 12:45 pm	**Özgür Sahin,** German Cancer Research Center (DKFZ) *Modulation of ERBB receptor-driven cell invasion and proliferation by miR-200c via targeting PLCγ1 and MIG-6 in breast cancer*
12:45 – 1:00 pm	**Johannes Schulte**, Universitätskinderklinik Essen, Germany *High ALK receptor tyrosine kinase expression prevail ALK mutation as a determining factor of an unfavorable phenotype in primary neuroblastoma*
1:00 – 3:00 pm	***Lunch Break and Poster Session I***
1:00 – 3:00 pm	**Company Satellite Lunch Sessions**
1:10 – 1:40 pm	**Dr. Richard Henfrey, Illumina UK Ltd** *Illumina's Array and Sequencing Tools for Genomic Discovery*
1.50 – 2.20 pm	**Roche Diagnostics GmbH**, *"A quantum leap in high throughput sequencing and realtime-PCR:"* **Dr. Dierk Evers, Roche Diagnostics GmbH,** Mannheim, Germany *The Light Cycler®1536 Real-Time PCR System: High throughput - redefined* **Dr. Guido Kopal, Roche Diagnostics GmbH**, Penzberg, Germany *Genome Sequencer FLX: Nex products and developments*
2.30 – 3.00 pm	**Raimo Tanzi,** Director, Business Development Next Generation Sequencing**, Applied Biosystems Europe**, Darmstadt, Germany *Whole Genome and Targeted Resequencing Using the SOLiD™ 3 Plus*

Symposium III: Animal, Cellular & Tissue Models

3:00 – 3:45 pm	**Keynote**
3:45 – 4:00 pm	**Bernhard G. Herrmann**, Max Planck Institute for Molecular Genetics, Berlin, Germany *Identification of Modifiers of Intestinal Tumor Formation and Progression utilising mouse Chromosome Substitution Strains*
4:00 – 4:15 pm	**Till Acker**, Institute of Neuropathology,University Giessen, Germany *A hypoxic niche regulates tumor stem cells.*
4:15 – 4:30 pm	**Deike Hesse**, German Institute of Human Nutrition, Potsdam-Rehbrücke *The role of the GTPase Arfrp1 for lipid droplet formation and the regulation of lipolysis in adipose tissue*
4:30 - 4:45 pm	**Guido Krebiehl**, Hertie Institute for Clinical Brain Research - Center of Neurology, University of Tuebingen, Germany *Mitochondrial dysfunction and impaired lysosomal degradation due to loss of Parkinson's disease associated protein DJ-1*
4:45 - 5:15 pm	***Coffee Break***

Symposium IV: Systems Biology

5:15 - 6:00 pm	**Keynote: Hans Westerhoff**, University of Manchester, UK; Vrije Universiteit Amsterdam, NL *Integrative Genomics with a little help from its friend: from genes to function and back*
6:00 - 6:15 pm	**Hans Lehrach**, Max Planck Institute for Molecular Genetics, Berlin, Germany *The TREAT1000 project: a step towards an individualisation of medicine*
6:15 - 6:30 pm	**Bodo Lange**, Max-Planck Institute for molecular Genetics, Berlin, Germany *A protein-protein interaction network links proteins of cell division pathways to their functional dysregulation in cancer*
6:30 - 6:45 pm	**Björn Schwanhäusser**, Max Delbrück Centrum Berlin, Germany *Genome-wide analysis of protein and mRNA half-lives reveals dynamic properties of mammalian gene expression*
6:45 - 7:00 pm	**Jörn Walter**, Saarland University, Germany *From Epigenetics to Epigenomics - DNA-methylation patterns along human chromosomes*
Evening Lecture	
7:00 - 8:00 pm	**Jens Reich**, Max-Delbrück Center, Berlin, Germany *Chimera Research in Development Biology - the Ethical Question*
8:00 - 10:00 pm	***Get-together (Wine, Cheese, Music)***

Saturday, November 28th, 2009

Symposium V: New Technologies

9:00 - 9:30 am	**Keynote I: Erich Wanker**, Max-Delbrück Center for Molecular Medicine, Berlin, Germany *Protein folding and misfolding: a major challenge for modern disease research*
9:30 - 10:00 am	**Keynote II: Jan Korbel**, EMBL, Heidelberg, Germany S*tructural Variations in the Genome- Using Next-Generation Sequencing to obtain insights into their Origins, Extent and Functional Impact*
10:00 - 10:15 am	**Hans-Jörg Warnatz**, Max Planck Institute for Molecular Genetics, Berlin, Germany *Cell Array-Based Functional Analysis of Human Chromosome 21 Gene Promoters*
10:15 - 10:30 am	**Andreas Schlicker**, Max Planck Institute for Informatics, Saarbrücken, Germany *Prioritizing Disease Genes from Association Studies using Functional Similarity*
10:30 - 10:45 am	**Mihail Sarov**, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany *TransgeneOmics: a platform for systems wide protein function analysis in metazoans*
10:45 - 12:45 pm	***Lunch Break and Poster Session II***

10:45 – 12:45 pm	**Company Satellite Lunch Sessions:**
10.55 – 11.25 am	**Dr. Nadine Schracke**, **Febit Biomed GmbH**, Heidelberg, Germany *The Power to Detect*
11.35 – 12.05 am	**Dr. Dirk Jürgensen, Affymetrix Europe** *Conquer the next wave of genomic discoveries: the next-generation Axiom™ Genotyping Solution from Affymetrix®*
12.15 – 12.45 pm	**Harry Boeltz, Fluidigm Europe B.V**., Netherlands *Library preparation for re-sequencing and long range sequencing made easy and robust using Fluidigm's proven nanofluidic technology*

Symposium VI: Transfer from Genomics to Application

12:45 - 1:30 pm	**Keynote: Jörg Rademann**, Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany *Virtual and dynamic small molecule screening in academic drug discovery*
1:30 - 1:45 pm	**Nikolaus Machuy**, MPI for Infection Biology, Berlin, Germany *Human host cell factors crucial for influenza virus replication identified by genome-wide RNAi screen*
1:45 - 2:00 pm	**Andreas Keller**, Biomarker Discovery Center Heidelberg, Germany *Specific miRNA fingerprints in patients' blood samples as novel diagnostic tool*
2:00 - 2:15 pm	**Ruprecht Kuner**, German Cancer Research Center, Heidelberg *Genome-wide analysis of deregulated microRNAs in prostate cancer*
2:15 - 2:30 pm	**Ulrike Korf,** German Cancer Research Center, Heidleberg *Integration of genomic and proteomic data to unwire chemoresistance mechanisms*
2:30 - 2:45 pm	**Patrick Linsel-Nitschke**, University of Lübeck, Germany *Risk prediction of myocardial infarction using a weighted genetic score - the German MI Family Study*
2:45 - 3:00 pm	**Ceremony: "Annemarie Poustka Poster Award of Medical Genome Research 2009" sponsored by Roche Diagnostics GmbH**
	Concluding Remarks: Hugo A. Katus, Universitätsklinik Heidelberg, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

Program (with speakers' biosketch)

Thursday, November 26th, 2009

1:00 – 4:00 pm	**Workshop: Next Generation Sequencing** (Org. Hans Lehrach, Ralf Sudbrak)
4:00 – 4:30 pm	***Coffee Break***
4:30 – 6:30 pm	**Workshop: Epigenetic Regulation** (Org. Peter Lichter)
7:00 pm	***Supper***

Friday, November 27th, 2009

Symposium I: Genomics of Common Disease

8:30 – 9:15 am

Opening Keynote Presentation

Genetics of myocardial infarction

Heribert Schunkert

Heribert Schunkert, MD is Professor of Cardiology and Director of the Department of Internal Medicine at Universitätsklinikum, Lübeck.
He is a graduate of Staatsexamen, West Germany and Rheinisch-Westfälisch Technische Hochschule, Aachen, FRG. Dr. Schunkert completed a research fellowship in medicine at Brigham and Women's Hospital, clinical fellowships in cardiology at Beth Israel Hospital and Universitätsklinikum, Regensburg, and interventional cardiology at Massachusetts General Hospital. He was appointed to the faculty at Harvard Medical School before returning to Universitätsklinikum, Regensburg, where he attained the rank of Professor.
Dr. Schunkert conducts research in the molecular biology and genetics of multifactorial cardiovascular disease and the induction of cellular growth by angiotensin II. He coordinates the EU sponsored project *Cardiogenics* and the BMBF sponsored project *Atherogenomics* within the NGFN to identify the genetic roots of myocardial infarction. He lists awards from the German Society of Cardiology, the German Society of Hypertension (has been elected in their board of directors), and the German Society of Preventive and Rehabilitative Medicine as well as the Travel-, Research- and Heisenberg Grants from the Deutsche Forschungsgemeinschaft and Research Grants from the Bundesministerium für Forschung and Technologie. Professional society memberships include the Deutsche Gesellschaft für Innere Medizin, Deutsche Gesellschaft für Kardiologie - Herz- und Kreislaufforschung and the International Society of Hypertension. Dr. Schunkert serves on the editorial boards of *Clinical and Experimental Hypertension*, *Cardiovascular Drugs and Therapy*, *Basic Research in Cardiology*, *Pharmacogenetics and Genomics, European Heart Journal, Circulation Cardiovascular Genetics* and is editor of *Cardiovasc*. He is the author of more than 300 publications in international journals.

9:15 – 9:30 am

The role of genetically determined metabotypes in the genetics of complex traits and polygenic disorders

Christian Gieger

studied Statistics from 1987 to 1993 at the Ludwig-Maximilians-Universität München where he received his PhD in 1998. Between 1993 and 1998 he worked as research associate, Chair of Statistics and its Applications with Prof. Dr. Ludwig Fahrmeir, Institute for Statistics at the University of Munich and between 1995 and 1999 at the Collaborative Research Center SFB 386. From 1999 to 2000 he worked in the industry for IBM and LION bioscience, Heidelberg and from 2002 to 2004 as Senior Scientist at the Fraunhofer Institute for Algorithms and Scientific Computing, Sankt Augustin. Since 2004 he is senior scientist and project leader at the Institute of Epidemiology of the Helmholtz Zentrum München and worked additionally until 2008 at the Chair of Epidemiology, IBE, Ludwig-Maximilians-Universität München. Since 2009 he acts as Head of the Research Unit 'Genetic Epidemiology' at the Helmholtz Zentrum München. He published on genome-wide association studies for various traits related to metabolic and cardiovascular diseases. His particular interest is focused on the genetics of intermediate traits and metabolic profiles (Metabolomics).

9:30 – 9:45 am

Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early onset extreme obesity in French and German study groups

Anke Hinney

PD Dr. rer. nat. Anke Hinney (*December 17th 1964 in Bielefeld). Biologist (1984–1990; Universities of Bielefeld, Tübingen and Sussex/UK). Research associate (1990 Korea Institute of Science and Technology/Seoul, South-Korea). PhD (1990–1993, Institute of Anthropology and Human Genetics, University of Tübingen); PostDoc (1993–1995, University of Düsseldorf). Head of molecular genetic laboratory of DFG-clinical research group (1995–2004 Department of Child- and Adolescent Psychiatry; University of Marburg). Since 2004 head of the research division ‘Molecular Genetics’ (Department of Child- and Adolescent Psychiatry; University of Duisburg-Essen). 2008 Habilitation for Molecular Genetics in Child- and Adolescent Psychiatry. Extensive expertise in the field of molecular genetic studies in complex disorders with a focus on childhood and adolescence (more than 130 peer reviewed original articles and reviews; several grants BMBF, NGFN, EU, DFG). Supervision of MD and PhD students.

9:45 – 10:00 am ***Genome-wide survey implicates CNVs in early-onset bipolar disorder***

Sven Cichon

Sven Cichon studied Biology at the University of Bonn and graduated in 1995 with a doctorate on the identification of genetic variability in CNS-expressed receptor/transporter genes and investigation of their impact for the development of neuropsychiatric disorders. During his post-doc time at the Institute of Human Genetics, University of Bonn, and at Millennium Pharmaceuticals Inc., Cambridge, MA, USA, his activities focused on disease gene identification for monogenic as well as genetically complex human diseases. Between 2001 and 2004, Dr. Cichon worked at the Department of Medical Genetics, University of Antwerp, Belgium, where he led a research group on psychiatric genetics. He returned to Bonn in 2004 to become Head of the Molecular Genetics Laboratory of the Department of Genomics at Life & Brain, a center of excellence in the field of translational biomedicine. The focus of his current research is on genomics strategies to unravel the molecular basis of complex diseases.

10:00 – 10:15 am ***Whole-genome, -exome, and targeted resequencing approaches to identify the remaining genetic heritability in Crohn's disease***

Andre Franke

Andre Franke studied Molecular Biology and Informatics and earned his doctorate at the Christian-Albrechts-University Kiel in 2006. Since then, he has been leading the genetics and bioinformatics group within the Institute of Clinical Molecular Biology (ICMB) of Prof. Dr. Stefan Schreiber in Kiel. He is also in charge of the high-throughput facilities at the ICMB, which are part of the national NGFN platform. In August 2008, Dr. Franke became Juniorprofessor for "Epithelial Barrier Diseases" within the DFG excellence cluster "Inflammation-at-Interfaces". In December 2008, Dr. Franke was awarded with the 100,000 Euro large "Hensel research prize".
Dr. Franke's main scientific interests are the development and establishment of novel high-throughput technologies, the inherent bioinformatic integration and the application of both to identify genetic and epigenetic causes of chronic inflammatory diseases like Crohn's disease, ulcerative colitis, psoriasis, primary sclerosing cholangitis, and atopic eczema. Having worked on genome-wide association studies for the last years, Dr. Franke's research agenda currently focuses on targeted enrichment strategies, whole-genome and whole-exome resequencing, and copy number variation analyses.

10:15 – 10:30 am ***MHC Haplotype Sequencing: An Integrated Approach to Common Disease***
Margret Hoehe

Dr. Margret Hoehe
Group Leader, Max Planck Institute for Molecular Genetics, Berlin
Education & Experience:
Studies of medicine and psychology, University of Munich, MD 1980, Dipl.Psych. 1983, PhD Neuroendocrinology/-pharmacology 1986; Psychiatric Hospital, University of Munich, 1982-1987; Clinical Neurogenetics Branch, National Institute of Mental Health,
1987-1992; Department of Genetics, Harvard Medical School (Laboratory of George Church), 1992-1994; Group Leader, Max Delbrück Center for Molecular Medicine, 1994-2002; CSO and co-founder, GenProfile, 1999-2002; from 2002 at the MPI-MG.
Focus of Research:
Early on "the introduction of the individual into science, both conceptually and concrete", from 1990 approaches to the systematic analysis of individual DNA sequence variation and its structure, including deep medical re-sequencing, haplotype-based approaches to disease gene discovery, and molecular haplotype sequencing in genes, genomic regions of interest and personal genomes.

10:30 – 11:00 am ***Coffee Break***

Welcome

11:00 – 11:30 am **Hans Lehrach,** Max Planck Institute for Molecular Genetics, Berlin, Germany

Thomas Rachel (MdB), Parlamentarischer Staatssekretär im Bundesministerium für Bildung und Forschung

Martin Hrabé de Angelis, Helmholtz Zentrum München, Speaker Project Committee of NGFN-Plus/NGFN-Transfer in the Program of Medical Genome Research

Symposium II: Genomics of Sporadic Cancer

11:30 – 12:15 pm

Opening Keynote Presentation

From genome-wide association studies to whole-genome sequencing in cancer

Ivo Gut

Ivo Gut graduated with a Ph.D in Physical Chemistry from the University of Basel (CH) in 1990. After post-docs at Harvard Medical School, Boston, USA and the Imperial Cancer Research Fund (London, UK) he joined the Department of Hans Lehrach at the Max-Planck-Institute for Molecular Biology (Berlin, D). In 1999 he moved to the Centre National de Génotypage (Paris, F) as Head of Technology Development. There he established the highest throughput genotyping operation in Europe. This platform has carried out more than 40 Genome Wide Association Studies and has a capacity of 70 billion genotypes per year. In the last three years he has established a 2nd generation DNA sequencing operation that is one of the largest in Europe and is deployed for the French contribution to the International Cancer Genome Consortium. In 2007 he was promoted to Associate Director of the CNG. He will be moving to Barcelona to direct the newly created "Centro Nacional de Analisis Genomico" in 2010.

12:15 – 12:30 pm

AML1-ETO collaborates with the HOX Gene Co-Factor, MEIS1 in inducing acute leukemia in the mouse bone marrow transplantation model

Vegi M. Naidu

Dr Naidu Vegi hails from Anakapalli, India and is currently working as post doctoral fellow in the Dr Christian Buske's group at the Institute of Experimental Tumor Research, University of Ulm. He has awarded PhD under Dr Buske's guidance in February 2009 with the topic "AML1-ETO and Meis1 collaborate to induce acute leukemia in mouse bone marrow transplantation model" as his dissertation.

Prior to joining Dr Buske's group, Dr. Vegi did his masters in Human Genetics at the Andhra University, India and then later joined as project assistant at the Centre for Cellular and Molecular Biology at Hyderabad, a premier research institute in India, where he worked in the field of population genetics. He has published four research articles in various international Journals.

Since his main interest was to pursue his doctoral studies in cancer biology he joined Dr Buske's Group and was involved in various projects besides his main PhD topic, reflected by several co-authorships in.

12:30 – 12:45 pm ***Modulation of ERBB receptor-driven cell invasion and proliferation by miR-200c via targeting PLCγ1 and MIG-6 in breast cancer***
Özgür Sahin

Özgür Sahin studied Molecular Biology and Genetics (B.Sc.) at Middle East Technical University (METU) in Ankara, Turkey. He then moved to University of Heidelberg where he received his M.Sc. degree from the International Molecular and Cell Biology Program. He obtained his PhD for his work on the systems level approach to identify novel drug targets in trastuzumab/Herceptin resistant breast carcinoma at German Cancer Research Center (DKFZ). During his PhD, he also developed an RNAi-based method to study cross-talk events in signaling pathways. Since September 2008, he is the group leader of RTK Signalling Group in the Division of Molecular Genome Analysis at DKFZ. His major research is focused on the role of miRNAs in drug resistance against targeted therapy- especially on the ErbB receptor targeted drugs- as well as in the metastasis process. Another focus of his group is to elucidate how the feedback regulation of highly oncogenic ErbB signaling is dysregulated in cancer.

12:45 – 1:00 pm ***High ALK receptor tyrosine kinase expression prevail ALK mutation as a determining factor of an unfavorable phenotype in primary neuroblastoma***
Johannes Schulte

Johannes H. Schulte attended medical school at the University of Essen, Germany, and at the University of Louisville, Kentucky. After graduation from medical school in 2005, he spent one year as a resident and postdoctoral researcher at the Dept. of Pathology, University of Bonn. Since 2006, he is a resident and postdoctoral research fellow at the University Children's Hospital Essen. His major focus is the molecular pathogenesis of the embryonal tumors neuroblastoma, retinoblastoma and medulloblastoma, mainly working on microRNAs, epigenetics and mouse models. Together with Zoltan Ivics from the Max Delbrück Center Berlin he is Principle Investigator of the NGFNplus Project "Transposon mutagenesis screen in mice to uncover the genetic basis of neuroblastoma and to identify neuroblastoma-initiating genes", and together with Alexander Schramm from the University Children's Hospital Essen he is Principle Investigator of the NGFNplus Project "The pathogenetic role and therapeutic potential of microRNAs in neuroblastoma".

1:00 – 3:00 pm ***Lunch Break and Poster Session I***

1:00 – 3:00 pm **Company Satellite Lunch Sessions**

1:10 – 1:40 pm **Dr. Richard Henfrey, Illumina UK Ltd**
Illumina's Array and Sequencing Tools for Genomic Discovery

1.50 – 2.20 pm **Roche Diagnostics GmbH**,
"A quantum leap in high throughput sequencing and realtime-PCR:"
Dr. Dierk Evers, Roche Diagnostics GmbH, Mannheim, Germany
The Light Cycler®1536 Real-Time PCR System: High throughput - redefined
Dr. Guido Kopal, Roche Diagnostics GmbH, Penzberg, Germany
Genome Sequencer FLX: Nex products and developments

2.30 – 3.00 pm **Raimo Tanzi,** Director, Business Development Next Generation Sequencing**, Applied Biosystems Europe**, Darmstadt, Germany
Whole Genome and Targeted Resequencing Using the SOLiD™ 3 Plus

Symposium III: Animal, Cellular & Tissue Models

3:00 – 3:45 pm **Keynote**

3:45 – 4:00 pm ***Identification of Modifiers of Intestinal Tumor Formation and Progression utilising mouse Chromosome Substitution Strains***

Bernhard G. Herrmann

Prof. Dr. Bernhard Herrmann is Director at the Max Planck Institute for Molecular Genetics in Berlin and Professor and Head of the Institute for Medical Genetics, Charité - CBF, Berlin. Dr. Herrmann did his Ph.D. work at the European Molecular Biology Laboratory in Heidelberg and received his doctorate from the University of Karlsruhe in 1987. He worked as EMBO postdoctoral fellow at the National Institute for Medical Research in London and was recruited by the Max-Planck-Institute for Developmental Biology as head of an independent junior research group in 1989. In 1994 he moved to the MPI for Immunobiology in Freiburg as senior scientist and head of a research group, before he was appointed as professor at the Charité and Max-Planck director in Berlin in 2003. Bernhard Herrmann is working in developmental genetics in the mouse with a strong focus on EMT, mesoderm formation and organogenesis, as well as on tumor formation and progression. He is an elected member of EMBO.

4:00 – 4:15 pm ***A hypoxic niche regulates tumor stem cells.***

Till Acker

University Training
1990-1996: Medical school in Freiburg, London/GB, San Diego/USA, Cape Town/SA
1993-1994: EUCOR Master of Clinical Research: Basel/CH, Freiburg, Strasbourg/F

Profession
1996-1999: Internship Neuropathology, University of Freiburg
1999-2001: Residency Neuropathology, University of Erlangen
2001-2003: Residency Neuropathology, University of Frankfurt
2003-2005: Postdoc, DFG, Dept. of CMB, Karolinska Institute, Stockholm/SE
2005-2008: Consultant of Neuropathology, University of Frankfurt
2006-2008: Max-Eder-Junior Research Group (Deutsche Krebshilfe)
since 2008: Professor (W3) of Neuropathology, University of Giessen

Scholarship/Prize
1993-1999: Stipendiary of the „Studienstiftung des deutschen Volkes“
1997: Boehringer-Ingelheim fellowship
2004: Keystone Symposium scholarship: „Biology of Hypoxia“, USA
2006: Neurowiss Prize; Frankfurt

4:15 – 4:30 pm ***The role of the GTPase Arfrp1 for lipid droplet formation and the regulation of lipolysis in adipose tissue***

Deike Hesse

Deike Hesse is a Ph.D. candidate of molecular biology at the German Institute of Human Nutrition (Deutsches Institut für Ernährungsforschung, DIfE). She studied biology at the University of Düsseldorf (Germany), Université de Nantes (France, ERASMUS/SOKRATES stipendiary) and at the University of Marburg (Germany) where she completed her diploma thesis on "Locomotor activity and diet-induced obesity in mice" in the department of animal physiology under the supervision of Prof. Dr. M. Klingenspor. Currently, Deike is working at the DIfE in the department of Experimental Diabetology under the supervision of Prof Dr. A. Schürmann as a third year Ph.D. candidate on the subject of the functional role of the Golgi-associated GTPase ARFRP1 in development and metabolism. Her particular research focus lies on the energy metabolism of the liver and the adipose tissue. She already presented her work during national and international scientific meetings and was granted two travel scholarships for attendance (grants by: Deutsche Diabetes-Gesellschaft and Potsdam Graduate School). Associated with her Ph.D. thesis Deike participated in a recent publication on the role of ARFRP1 for the targeting of E-Cadherin as a co-author.

4:30 - 4:45 pm ***Mitochondrial dysfunction and impaired lysosomal degradation due to loss of Parkinson's disease associated protein DJ-1***

Guido Krebiehl

Dr. Guido Krebiehl, born 1973 in Pforzheim, Germany
2002 diploma in biology with a main emphasis on genetics at the University of Hohenheim, Stuttgart, Germany
2007 Ph.D. at the Institute for Radiobiology and Molecular Environmental Research (Head: Prof. Dr. H.-P. Rodemann), Department of Radiation Oncology, University of Tübingen, Germany,Topic: *„Analyse relevanter Signalwege der strahleninduzierten COX-2 Expression in Tumorzellen"*
2007- present Postdoctoral Fellowship at the Laboratory for Functional Neurogenomic (Head: Prof. Dr. R. Krüger), Centre of Neurology and Hertie-Institute for Clinical Brain Research, University of Tübingen, Germany
Current research focus: Mitochondrial dynamics in neurodegeneration in Parkinson's disease

4:45 - 5:15 pm ***Coffee Break***

Symposium IV: Systems Biology

5:15 - 6:00 pm

Opening Keynote Presentation

Integrative Genomics with a little help from its friend: from genes to function and back

Hans Westerhoff

Hans Westerhoff has long been active in Systems Biology, integrating experimental and theoretical activities, in search of the network principles behind biological function. One of his long-term visions is the silicon human, an experiment based mathematical model of the human at continuously increasing accuracy. After staff positions at the (US) NIH and the Netherlands Cancer Institute, Westerhoff became Professor of Microbial Physiology at the VU University Amsterdam and Professor of Mathematical Biochemistry at the University of Amsterdam. Recently, he also became the AstraZeneca-funded Professor of Systems Biology at the University of Manchester (70 %), Director of the Manchester Centre for Integrative Systems Biology and Director of the Doctoral Training Centre for Systems Biology at Manchester. Westerhoff is on various boards of Systems Biology programs in Germany, the UK and Luxembourg, and has also been the main organizer of the FEBS Advanced Courses on Systems Biology.

6:00 - 6:15 pm

The TREAT1000 project: a step towards an individualisation of medicine

Hans Lehrach

Hans Lehrach studied Chemistry at the University of Vienna and accomplished his Ph.D. in 1974 at the MPI for Experimental Medicine and at the MPI for Biophysical Chemistry in Göttingen. He was a research fellow at Harvard University, Boston. After his return to Europe he took up position as the head of a research group at the EMBL, Heidelberg, and later on as head of the Department of Genome Analysis at the ICRF, London. In 1994, Hans Lehrach took up the post of Director at the Max Planck Institute for Molecular Genetics in Berlin and heads the Department of Vertebrate Genomics. At the same time he holds a Professorship at the faculty of Biochemistry at the Free University.

Hans Lehrach was a speaker of the German Human Genome Project and is currently a member of the project committee of the National Genome Research Network (NGFN). Moreover, he is e.g. a member of the European Molecular Biology Organization (EMBO) and of the scientific advisory board of the Austrian Genome Research Project (GENAu), as well as of Editorial boards of several scientific journals.

In 1993 Hans Lehrach was a Fellow of the American Association for the Advancement of Science, he received the Ján Jessenius SAS Medal of Honour for outstanding achievements in medical sciences of the Slovak Academy of Sciences (2003, Bratislava) and the Karl Heinz Beckurts Award for achievements in genome research (2004, Munich).

6:15 - 6:30 pm

A protein-protein interaction network links proteins of cell division pathways to their functional dysregulation in cancer

Bodo Lange

Bodo Lange studied Biology at the University of Bonn (Germany), University of Kent (UK) and at the TU Munich (Germany). Subsequently he carried out his PhD at the University of Manchester (UK) followed by postdoctoral work at the Kansai Advanced Research Centre in Kobe (Japan) and at the European Molecular Biology Laboratory (EMBL) in Heidelberg studying the regulation of the cell cycle throughout development in *Drosophila*. He habilitated and became lecturer in 2003 then joining the department of Prof. Hans Lehrach at the Max Planck Institute for Molecular Genetics in Berlin as a group leader. His group investigates the molecular composition and function of protein complexes that are key components in cell division and proliferation pathways relevant for cancer and neurodegenerative disease progression. Bodo Lange is coordinator of IG MUTANOM that develops a Systems Biology model for the prediction of the functional consequence of cancer mutations with the aim to translate this knowledge into the clinic for improving the diagnosis and therapy of cancer.

6:30 - 6:45 pm

Genome-wide analysis of protein and mRNA half-lives reveals dynamic properties of mammalian gene expression

Björn Schwanhäusser

Since April 2007 I am a PhD student in the group of Matthias Selbach at the Max-Delbrück-Centrum for Molecular Medicine (MDC) in Berlin studying global protein translation using quantitative mass spectrometry. Between 2001 and 2006 I was studying Biology at the Technical University Darmstadt. During my diploma thesis at the MPI for Infection Biology in Berlin I was investigating the underlying molecular mechanism in persistent *Salmonella* infections.

6:45 - 7:00 pm

From Epigenetics to Epigenomics - DNA-methylation patterns along human chromosomes

Jörn Walter

NAME: Dr. Jörn Erik Walter
PLACE AND DATE OF BIRTH: Eslohe/Germany 04.05.1958
NATIONALITY: German
PRESENT POSITION: Chair of Genetics, Full Professor
EDUCATION: 1977 High School Graduation (Abitur)
1977-1986 University Study (Biology and History)
1987 Grad. in Biology
1990 PhD in Biology
DATE / DEGREE/ UNIVERSITY: 1987 Diplom Biology, Freie Universität Berlin
DATE / POSTGRADUATE (PhD): 1990 Dr. rer. nat. (Biology), FU Berlin
DATE / POSTDOCTORAL: 1990 Postdoc, MPI Berlin
1992-94 Postdoc Cambridge, UK
1994-2000 Group leader, MPI Berlin
APPOINTMENTS:
Full Professor, Chair of Genetics, Saarbrücken (since 2000)
Scientific Advisor, Epigenomics AG, Berlin/Seattle (since 2001);
Coordiantor of the DFG SPP1129 “Epigenetics”;
Member of the FP6 NoE “The Epigenome”;
Member of the SPP 1356
“Pluripotency and Reprogramming“
AWARDS: EMBO and EU- Fellowships

7:00 - 8:00 pm

Evening Lecture

Chimera *Research in Developmental Biology – the Ethical Question*

Jens Reich

Jens Reich graduated in medicine from Humboldt University (then East-Berlin, GDR) in 1962 and defended his M.D. in 1964 at the same university. He then studied biochemistry at Jena University (1964-1968). From 1968 he worked as group leader at the Biology and Medicine Institutes of the East-German Academy of Sciences. He specialized in mathematical modelling of biochemical and cellular processes and published, together with E.E. Selkov (USSR) as co-author, a theoretical treatise ”Energy Metabolism of the Cell” (Academic Press, 1981). In 1976 he defended his habilitation degree in theoretical cell biology and became professor of Biomathematics in 1979.

In the 80ies he was degraded as research group leader because of his oppositional activities in private and public grass-root political groups. He used his isolation for a start in the new field of molecular bioinformatics which became important in the late 80ies.

Reich was one of the co-founders of the grass-root movement “New Forum” in 1989. With the collapse of the communist GDR regime he was elected as member of the democratic last parliament. With unification of Germany in October 1990 the Academy of Sciences was dissolved. Reich accepted a visiting professorship at the German Cancer Research Center and another one at Harvard University.

In 1992, with the foundation of the Max-Delbrück-Center of Molecular Biology Reich founded a research group in genomic bioinformatics and took part with a research project in the German Genome Research Programme (“Association study of genomic variants with risk status in cholesterol metabolism of healthy persons”), for several years as speaker of the whole programme. From 1998 until 2004 he was full professor of biomathematics at Humboldt University.

At present Reich is a member of the Berlin-Brandenburg Academy of Sciences and of the German Ethics Council, representing here the scientific community in bioethical discussions. He takes part in the HepatoSys research programme as project leader “Human Iron Metabolism”, together with research groups at Heidelberg University and EMBL.

8:00 - 10:00 pm ***Get-together (Wine, Cheese, Music)***

Saturday, November 28th, 2009

Symposium V: New Technologies

9:00 - 9:30 am

Opening Keynote I Presentation

Protein folding and misfolding: a major challenge for modern disease research

Erich Wanker

Prof. Dr. Erich E. Wanker is head of Neuroproteomics at the Max Delbrueck Center for Molecular Medicine, Berlin Buch. After his diploma and PhD from the Technical University of Graz, Austria, and a postdoctoral fellowship in the lab of Prof. David Meyer at the University of California, Los Angeles, he became a group leader in the department of Prof. Hans Lehrach at the Max Planck Institute for Molecular Genetics, Berlin Dahlem (1995). There, he discovered polyglutamine-mediated protein misfolding and aggregation in the pathomechanism of Huntington's disease. He extended his research to other protein misfolding disorders and discovered small molecule modulators of Alzheimer's and Parkinson's disease. His group also belongs to the international leaders in network biology, pioneering the field with the generation of the first protein interaction networks of the human proteome. He has won the BioFuture research award (BMBF, 1998), the Minna-James-Heinemann award (2000), the GO-Bio award (BMBF, 2006) and the Erwin Schrödinger award of the Helmholtz Association (2008). Currently, he is coordinator of the NGFNplus IG NeuroNet.

9:30 - 10:00 am

Opening Keynote II Presentation

Structural Variations in the Genome –Using Next-Generation Sequencing to Obtain Insights into their Origin, Extent, and Functional Impact

Jan Korbel

Ph.D. 2005, EMBL Heidelberg, Humboldt University Berlin.
Postdoctoral research at Yale University, New Haven, CT, with Mike Snyder and Mark Gerstein
Group Leader at EMBL since October 2008, in the Genome Biology Unit
Joint appointment with the EMBL-EBI.

10:00 - 10:15 am ***Cell Array-Based Functional Analysis of Human Chromosome 21 Gene Promoters***

Hans-Jörg Warnatz

Hans-Jörg Warnatz grew up in southern Germany, in the Forest of Odes. After a short detour to the Technical University of Karlsruhe, studying Informatics, he changed to Molecular and Cell Biology in Heidelberg. He finished his Diploma at the Max Planck Institute for Medical Research next to the Neckar river, investigating the biochemistry of an unconventional myosin in the soil-living amoebum *Dictyostelium discoideum*.
In 2002, Hans-Jörg moved to Berlin and started the work for his PhD in the Max Planck Institute for Molecular Genetics. His thesis reported high-throughput functional analyses of the proteins encoded on human chromosome 21, investigating subcellular localizations and protein interactions. His current projects in the group of Dr. Marie-Laure Yaspo focus on the interplay between human transcription factors and gene promoters. He is applying new technologies (cell arrays, ChIP-seq) for chromosome- and genome-wide studies of regulatory networks driving gene transcription.

10:15 - 10:30 am ***Prioritizing Disease Genes from Association Studies using Functional Similarity***

Andreas Schlicker

Andreas Schlicker received his master's degree in bioinformatics from Saarland University, Saarbrücken, Germany, in 2005. Since then he has been a research scientist in the group for Molecular Networks in Medical Bioinformatics (http://www.medbioinf.de) headed by Dr. Mario Albrecht within the Department for Computational Biology and Applied Algorithmics directed by Prof. Dr. Dr. Thomas Lengauer at the Max Planck Institute for Informatics in Saarbrücken. Currently, he is finishing his PhD studies, which involved the development of novel methods for analyzing the semantic and functional similarity of genes based on their ontological annotation, the implementation of new tools for the efficient computation of similarity measures, and the application of the latter to molecular interaction networks and the prioritization of candidate disease genes. His scientific interests include the integration and analysis of large-scale biological datasets with a particular focus on human systems biology and medicine.

10:30 - 10:45 am ***TransgeneOmics: a platform for systems wide protein function analysis in metazoans***

Mihail Sarov

We have developed a scalable, high throughput, serial-parallel DNA engineering approach based on *in vivo* recombineering *in E. coli*. The method is independent on the presence of restriction sites, does not require DNA isolation and *in vitro* manipulation and can be applied to very large constructs >200Kbp, allowing genes to be modified within their endogenous genomic context. We are using this technology to build genome scale transgenic resources for the main metazoan model systems (cultured mammalian cells*, C. elegans* and *Drosophila*), which will make any protein amenable to generic, tag-based protein purification and localization analysis.
Since 2008 TransgeneOme Project leader

2007-2008 Postdoc in the lab of Tony Hyman, Max Planck institute of Molecular Cell Biology and Genetics
2002-2006 PhD in the lab of Francis Stewart, Biotec Center, TUD
1999-2001 Research scientist at Institute of Molecular Biology, Bulgarian Academy of Sciences
1994-1999 M.Sc. in Molecular Biology, Sofia University

10:45 - 12:45 pm ***Lunch Break and Poster Session II***

10:45 – 12:45 pm **Company Satellite Lunch Sessions:**

10.55 – 11.25 am **Dr. Nadine Schracke**, **Febit Biomed GmbH**, Heidelberg, Germany
The Power to Detect

11.35 – 12.05 am **Dr. Dirk Jürgensen, Affymetrix Europe**
Conquer the next wave of genomic discoveries: the next-generation Axiom™ Genotyping Solution from Affymetrix®

12.15 – 12.45 pm **Harry Boeltz, Fluidigm Europe B.V.**, Netherlands
Library preparation for re-sequencing and long range sequencing made easy and robust using Fluidigm's proven nanofluidic technology

Symposium VI: Transfer from Genomics to Application

12:45 - 1:30 pm ***Opening Keynote Presentation***

Virtual and dynamic small molecule screening in academic drug discovery

Jörg Rademann

Professor for Medicinal Chemistry
Curriculum vitae
Scientific Education:
1988-1993 Studies in Chemistry and Biochemistry at the Universities Konstanz and the graduate school of Rutgers University, USA
1993-1994 Diplomarbeit at the University of Konstanz: "On the synthesis of O-linked glycopeptides" (very good)
1994-1997 PhD Studies at the University of Konstanz with R. R. Schmidt: "A method for the solid phase synthesis of oligosaccharides" (summa cum laude)
Academic Appointments:
1997 – 1999 Postdoctoral Fellow at Carlsberg Laboratory and the Center for Solid Phase Organic Combinatorial Chemistry
1999-2004 Leader of the DFG- junior research group for diversity-oriented synthesis and solid phase technology at Tübingen University
Lecturer in the graduate college "Chemistry in Interphases"
2003 Habilitation, venia legendi in organic chemistry
since 2004 Professor of Medicinal Chemistry at Free University Berlin
Head of Medicinal Chemistry at the FMP (Leibniz Research Institute of Molecular Pharmacology)
2008 Call on the chair for Medicinal Chemistry, Leipzig University

1:30 - 1:45 pm ***Human host cell factors crucial for influenza virus replication identified by genome-wide RNAi screen***

Nikolaus Machuy

Nikolaus Machuy is head of the automated RNA interference screening platform at the Max-Planck-Institute for Infection Biology in Berlin. In addition he is a consultant for RNAx, a biotech company providing RNAi-based services.
Nik Machuy studied Biology at the Universities Gießen and Potsdam, where he got his Diploma in 1998. From 1998-2001 he did his PhD at the MPIIB. 2002 he started to set up the RNAi screening platform at the MPIIB. From 2003-2005, he worked as a team leader for RNAx. Since 2005, he holds his current position.

1:45 - 2:00 pm ***Specific miRNA fingerprints in patients' blood samples as novel diagnostic tool***

Andreas Keller

Dr. Andreas Keller is project leader of the Heidelberg Biomarker Discovery Center (BDC), a part of the Biotech Cluster Rhine-Neckar. In 2008, Andreas joined febit biomed GmbH, a biotech company located in Heidelberg, Germany and Boston, Massachusetts, USA. First, he was responsible for the Analytical Services Department and bioinformatics method development before moving into the role as head of the Genomics Service department. His primary focuses of research in this position are in network-based analysis of high-throughput data as microarrays, non-invasive cancer and disease diagnosis and the evaluation of so-called Next Generation Sequencing data.
Before joining febit, Dr. Keller finished his Ph.D. at the Center of Bioinformatics at Saarland University, studying highly sensitive and specific not-invasive disease diagnosis of human malignancies (including cancer). His thesis was supervised by Professor Hans-Peter Lehnhof. Dr. Keller also holds a Masters degree in Computational Biology from Saarland University

2:00 - 2:15 pm ***Genome-wide analysis of deregulated microRNAs in prostate cancer***

Ruprecht Kuner

Ruprecht Kuner studied biology at the Eberhard Karls University in Tübingen (1991-1998) with a focus on human genetics and virology. He received his Ph.D. in molecular cancer biology at the Humboldt University Berlin (2002). His thesis aimed the systematic identification and molecular characterization of genes deregulated in gynecological cancer. Since 2003, he works as a scientist in the Division of Molecular Genome Analysis at the German Cancer Research Center. Based on the knowledge that the overall gene expression activity in cells and tissues is orchestrated by the complex networking of many different cellular pathways, a first goal was to identify denominator genes involved in human diseases like cardiovascular and cancer diseases. The present work includes diverse OMICS technologies and cellular models for the systematic analysis of prostate and lung cancer. The integration of different molecular levels like genomic, transcript, microRNA and protein

data aims to identify robust diagnostic and prognostic signatures, and may harbor therapeutic applications.

2:15 - 2:30 pm ***Integration of genomic and proteomic data to unwire chemoresistance mechanisms***

Ulrike Korf

Ulrike Korf received a PhD in Organic Chemistry from the University of Hamburg in 1993. From 19993 to 1998 she was a Postdoctoral Fellow at the Howard Hughes Medical Institute, Department of Biochemistry, and at the Department of Immunology at the University of Washington in Seattle, USA. After a two-year period in Science Management in she joined the Division of Annemarie Poustka at the DKFZ in Heidelberg to set-up a Protein Chemistry lab. Since 2004 she is heading her own research group focussing on quantitative protein microarray-based proteomics as required by systems biology applications as well by translational research.

2:30 - 2:45 pm ***Risk prediction of myocardial infarction using a weighted genetic score - the German MI Family Study***

Patrick Linsel-Nitschke

Dr. med. Patrick Linsel-Nitschke
Internist und Kardiologe, Oberarzt der Medizinischen Klinik II, Universitätsklinikum Schleswig-Holstein, Campus Lübeck
Forschungsschwerpunkte: Genetik des Myokardinfarktes, Genetik des Lipidstoffwechsels, Monozyten- uns Makrophagen-Transkriptom
Wissenschaftliche Ausbildung:
Seit 2005: Mitarbeit im Molekulargenetischen Labor der Medizinischen Klinik II, Universität Lübeck (Prof. Heribert Schunkert/ Prof. Jeanette Erdmann)
2001-2005 Postdoctoral Fellow, Division of Molecular Medicine, Department of Medicine, College of Physician and Surgeons, Columbia University, New York (Prof. Alan Tall)
1998 bis 2001: Weiterbildung Innere Medizin an der Medizinischen Kernklinik, Universitätskrankenhaus Hamburg-Eppendorf (Prof. Heiner Greten). Wissenschaftliche Tätigkeit und Promotion im Biochemischen Stoffwechsellabor (Prof. Ulrike Beisiegel)

2:45 - 3:00 pm **Ceremony: "Annemarie Poustka Poster Award of Medical Genome Research 2009" sponsored by Roche Diagnostics GmbH**

Concluding Remarks: Hugo A. Katus, Universitätsklinik Heidelberg, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

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Overviews

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Oral Presentations

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91	O-1-1	Christian Gieger	The role of genetically determined metabotypes in the genetics of complex traits and polygenic disorders.	
92	O-1-2	Andre Franke	Whole-genome, -exome, and targeted resequencing approaches to identify the remaining genetic heritability in Crohn's disease	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
93	O-1-3	Sven Cichon	Genome-wide survey implicates CNVs in early-onset bipolar disorder	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
94	O-1-4	Anke Hinney	Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early onset extreme obesity in French and German study groups	IG Molecular Mechanisms in Obesity
95	O-1-5	M.R. Hoehe	MHC Haplotype Sequencing: An Integrated Approach to Common Disease	IG MHC Haplotype Sequencing: An Intergrated Approach to Common Disease
Symposium II - Genomics of Sporadic Cancer				
98	2-Keynote	Ivo Gut	From genome-wide association studies to whole-genome sequencing in cancer	
99	O-2-1	Vegi M. Naidu	AML1-ETO colaborates with the HOX Gene Co-factor MEIS1 in inducing acute leukemia in the mouse bone marrow transplantation model	Genomics of Acute Leukemias
100	O-2-2	Özgür Sahin	Modulation of ERBB receptor-driven cell invasion and proliferation by miR-200c via targeting PLC?1 and MIG-6 in breast cancer	IG Cellular Systems Genomics in Health and Disease
101	O-2-3	Johannes H. Schulte	High ALK receptor tyrosine kinase expression prevail ALK mutation as a determining factor of an unfavorable phenotype in primary neuroblastoma	IG Neuroblastoma Genome Interaction Network
Symposium III - Animal, Cellular & Tissue Models				
105	O-3-1	Bernhard G. Herrmann	Identification of Modifiers of Intestinal Tumor Formation and Progression utilising mouse Chromosome Substitution Strains	IG Modifiers of Intestinal Tumor Formation and Progression
106	O-3-2	Till Acker	A hypoxic niche regulates tumor stem cells.	
107	O-3-3	Annette Schürmann	The role of the GTPase Arfrp1 for lipid droplet formation and the regulation of lipolysis in adipose tissue	IG Molecular Mechanisms in Obesity
108	O-3-4	Guido Krebiehl	Mitochondrial dysfunction and impaired lysosomal degradation due to loss of Parkinson's disease associated protein DJ-1	

Page	Abstract	Submitting Author	Abstract Title	Consortium
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113	O-4-1	Hans Lehrach	The TREAT1000 project: a step towards an individualisation of medicine	IG Mutanom
114	O-4-2	Bodo M.H. Lange	A protein-protein interaction network links proteins of cell division pathways to their functional dysregulation in cancer	IG Systems Biology of Genetic Diseases (Mutanom)
115	O-4-3	Björn Schwanhäusser	Genome-wide analysis of protein and mRNA half-lives reveals dynamic properties of mammalian gene expression	IG Neurodegenerative Diseases Networks (NeuroNet)
116	O-4-4	Jörn Walter	From Epigenetics to Epigenomics - DNA-methylation patterns along human chromosomes	IG Modifiers of Intestinal Tumor Formation and Progression
117	Evening Lecture	Jens Reich	Chimera Research in Development Biology - the Ethical Question	
Symposium V – New Technologies				
121	5-Keynote I	Erich Wanker	*Protein folding and misfolding: a major challenge for modern disease research*	IG NeuroNet
122	5-Keynote II	Jan Korbel	*Structural Variations in the Genome- Using Next-Gen Sequencing to obtain insights into their Origins, Extent and Functional Impact*	
123	O-5-1	Hans-Jörg Warnatz	Cell Array-Based Functional Analysis of Human Chromosome 21 Gene Promoters	
124	O-5-2	Andreas Schlicker	Prioritizing Disease Genes from Association Studies using Functional Similarity	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
125	O-5-3	Mihail Sarov	TransgeneOmics: a platform for systems wide protein function analysis in metazoans	IG From Disease Genes to Protein Pathways (DiGTOP)
Symposium VI -Transfer from Genomics to Application				
128	6-Keynote	Jörg Rademann	*Virtual and dynamic small molecule screening in academic drug discovery*	
129	O-6-1	Andreas Keller	Specific miRNA fingerprints in patients' blood samples as novel diagnostic tool	
130	O-6-2	Nikolaus Machuy	Human host cell factors crucial for influenza virus replication identified by genome-wide RNAi screen	
131	O-6-3	Ulrike Korf	Integration of genomic and proteomic data to unwire chemoresistance mechanisms	IG Cellular Systems Genomics in Health and Disease
132	O-6-4	Ruprecht Kuner	Genome-wide analysis of deregulated microRNAs in prostate cancer	IG Integrated Genome Network of Prostate Cancer
133	O-6-5	Patrick Linsel-Nitschke	Risk prediction of myocardial infarction using a weighted genetic score--the German MI Family Study	IG Genomics of Atherosclerosis

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List of Poster Abstracts

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All posters will be displayed continuously throughout the duration of the meeting. Autors will be present at their posters for discussion during the designated time.

Poster Session I: Even numbers
Friday, November 27th 2009
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140	P-1-2	Jan G. Hengstler	ERBB2 induces an anti-apoptotic expression pattern of Bcl-2 family members in node negative breast cancer	IA Breast Cancer Kit
141	P-1-3	Valentina Vladimirova	Epigenetic silencing of a Lim homeobox transcription factor LHX9 modulates the invasive behaviour of malignant gliomas of childhood	IG Brain Tumor Network
142	P-1-4	Jitao David Zhang	flowDeconvolutor: A reproducible and flexible approach to flow cytometric cell cycle analysis	IG Cellular Systems Genomics in Health and Disease
143	P-1-5	Yvonne G. Weber	A GLUT1 mutation in patients with spastic paraplegia and paroxysmal dyskinesia	IG Epilepsy and Migraine Integrated Network (EMINet)
144	P-1-6	Holger Trucks	Recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 predispose to idiopathic generalised epilepsies	IG Epilepsy and Migraine Integrated Network (EMINet)
145	P-1-7	S Maljevic	Biophysical characterization of a T-type Ca channel mutation and an HCN4 polymorphism found in a family with idiopathic generalized epilepsy	IG Epilepsy and Migraine Integrated Network (EMINet)
146	P-1-8	Katharina Pernhorst	Allelic promoter variants accumulated in epileptic patients: indication for mRNA-expression alterations of corresponding genes	IG Epilepsy and Migraine Integrated Network (EMINet)
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148	P-1-10	Tobias Freilinger	Genome-wide association study in migraine without aura	IG Epilepsy and Migraine Integrated Network (EMINet)
149	P-1-11	Guntram Borck	High-density SNP array linkage and CNV analyses in familial forms of common migraine	IG Epilepsy and Migraine Integrated Network (EMINet)
150	P-1-12	Costin Leu	GENOME-WIDE ASSOCIATION MAPPING OF SUSCEPTIBILITY ALLELES PREDISPOSING TO IDIOPATHIC GENERALISED EPILEPSY	IG Epilepsy and Migraine Integrated Network (EMINet)
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153	P-1-15	Christine Klein	Role of single heterozygous PINK1 mutations as a susceptibility factor in Parkinson disease: Evidence from a family study including clinical and neuroimaging investigations	IG Functional Genomics of Parkinson
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157	P-1-19	Michael Wilk	Promoter analyses of Alzheimer´s disease-related genes ADAM10, BACE1 and APP	IG Gene Identification and Functional Analyses in Alzheimer's Disease
158	P-1-20	Jan Bieschke	EGCG is a potent remodeling agent of mature amyloid fibrils and reduces cellular toxicity	IG Gene Identification and Functional Analyses in Alzheimer's Disease
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173	P-1-35	N. Tyshchenko	Definition of the critical region in chromosome band 1q25 for patients with mental retardation and cleft lip or palate.	IG German Mental Retardation Network (MRNET)
174	P-1-36	Markus Zweier	Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a relatively frequent cause of severe mental retardation and diminish MECP2 expression	IG German Mental Retardation Network (MRNET)
175	P-1-37	Karl Hackmann	MRNET TP7 – Summary of high resolution molecular karyotyping of patients with mental retardation – Identification of regions containing candidate genes	IG German Mental Retardation Network (MRNET)
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177	P-1-39	Juliane Hoyer	Five new families with 15q13 microdeletion: Further delineation of the phenotypical spectrum and review of the literature	IG German Mental Retardation Network (MRNET)
178	P-1-40	Kristin Hofmann	Detection of a 7Mb de novo deletion in a patient with distal Athrogryposis Type 2B (DA2B)	IG German Mental Retardation Network (MRNET)
179	P-1-41	N. Tyshchenko	MOMO syndrome: an obesity mental retardation syndrome and further search for the disease causing mutation	IG German Mental Retardation Network (MRNET)
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181	P-1-43	Alexander Götz	Carbon nanoparticle (CNP) induced transient pulmonary inflammation in mice: indications of IL1B, IL18 and VEGF cascade in maintenance of homeostasis	IG German Mouse Clinic - Deciphering Comorbidity in Human Disease Models
182	P-1-44	Kateryna Micklich	MVD013 a mouse model of inherited polycythaemia	IG German Mouse Clinic - Deciphering Comorbidity in Human Disease Models
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192	P-1-54	Marc Zapatka	Identifying disease associated SNPs using biological pathway knowledge	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
193	P-1-55	Susanne Lucae	The neuronal transporter gene SLC6A15 confers risk to major depression	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
194	P-1-56	René Breuer	REFINED PHENOTYPING REGARDING GABAA RECEPTOR GENES AND THEIR IMPACT ON BIPOLAR DISORDER	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
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Symposium I

Genomics of Common Disease

Genetics of myocardial infarction

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A small region on chromosome 9p21.3, discovered in parallel by three groups in the year 2007, turned out to be prototypical for modern genetics of myocardial infarction. The risk allele is frequent, acts independently of traditional risk factors, and confers a modest yet highly reproducible hazard. Since then another 12 chromosomal regions have been identified to affect the risk of myocardial infarction or coronary artery disease. While the number of risk alleles is growing rapidly, several conclusions can be drawn from these initial findings. Firstly, it appears that multiple hitherto unknown molecular mechanisms - initiated by these chromosomal variants - ultimately precipitate atherosclerotic sequellae. Secondly, essentially all Caucasians carry a variable number of risk alleles such that disease manifestation is affected to a lesser or larger extent by these inherited factors in basically all individuals. Once the functional genomic mechanisms are understood, this emerging field may offer novel opportunities to neutralize a broadly found genetic susceptibility for coronary artery disease. In parallel, the newly discovered genes will open novel opportunities for disease prediction. In summary, modern myocardial infarction genetics carry the promise to identify individuals at high risk and to improve prevention and therapy of this dreadful disease.

The role of genetically determined metabotypes in the genetics of complex traits and polygenic disorders.

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Serum metabolite concentrations provide a direct readout of biological processes in the human body, and are associated with disorders such as cardiovascular and metabolic diseases. We have previously identified frequent genetic polymorphisms with large effects sizes that alter an individual's metabolic capacities (Gieger et al., PLoS Genetics, 2008). We argue that knowledge of these "genetically determined metabotypes" in the human population is key to identifying the contributions and interaction of genetic and environmental factors in the etiology of complex diseases. Here we present a genome-wide association study with 163 metabolic traits, covering a biologically relevant panel of amino acids, sugars, acylcarnitines, and phospholipids, using 1809 participants from the KORA population, which we replicated in the TwinsUK cohort with 422 participants. Most often, the genetic variant is located in or near enzyme or solute carrier coding genes, where the associating metabolic traits match the proteins' function, many of these polymorphisms being located in rate limiting steps of important enzymatic reactions. For several loci, an association with clinical endpoints has previously been reported, such as SLC22A4 with Crohn's disease, FADS1 with hyperactivity and cholesterol/triglyceride levels, and ACADS with susceptibility to ethylmalonic aciduria. Here we report association of these loci with different metabolic phenotypes and show that these allow deriving new functional information about the underlying pathophysiology. In summary, this study allowed us to draw a systemic perspective of the genetic variation that is found in human metabolism. In contrast to most GWAS with clinical endpoints, it appears that for metabolic traits most of the associations are linked to genetic variants in genes with a matching metabolic function. Our study shows the exciting potential of Metabolomics to unravel the genetics of complex traits and polygenic disorders.

Whole-genome, -exome, and targeted resequencing approaches to identify the remaining genetic heritability in Crohn's disease

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During the last 4 years, hundreds of genetic susceptibility loci were discovered by means of genome-wide association studies (GWAS) for various complex phenotypes, including the chronic inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC). Although most of the GWAS findings have significantly improved our knowledge of the pathophysiology of the respective diseases, the identified variants only explain a fraction of the estimated heritabilities. For example, for Crohn's disease, one of the diseases where GWAS were most successful, the more than 30 identified genetic risk loci still explain less than 10% of the estimated heritability. Recently, a hallmark publication has pointed out potential sources of the so-called "missing heritability". It is speculated that much of this "missing heritability" can be attributed to rare variants (frequency <5% in population). Rare variants are and were insufficiently represented by available genome-wide SNP arrays as well as in the HapMap resource. Therefore, much attention is now on the public 1000 Genome project as well as Next Generation resequencing projects in general.
In the presentation, a brief overview on the current knowledge of IBD genetics is given, while the main part of the presentation will cover our most recent results of targeted, whole-exome (45 exomes completed), and whole-genome (1 full patient genome available) resequencing in CD employing Next Generation Sequencing technology.

Genome-wide survey implicates CNVs in early-onset bipolar disorder

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We used genome-wide single-nucleotide polymorphism (SNP) data to screen for the presence of copy number variants (CNVs) in 882 patients with bipolar disorder (BD) and 872 controls; 290 (33%) of the patients had an early age-at-onset of =21 years. We systematically filtered for CNVs covering >30 markers and directly affecting at least one RefSeq gene. We tested whether a) the genome-wide burden of CNVs differed between patients and controls and b) the frequency of specific CNVs was different between patients and controls. Burden analysis revealed that the frequency and size of CNVs did not differ significantly between all patients and controls. Separate burden analyses of patients with an age-at-onset =21 years (AO=21y) and age-at-onset >21 years (AO>21y), however, showed that the frequency of microduplications was higher in patients with an AO=21y compared to controls (P=0.00022) and that the average size of singleton microdeletions was larger in AO=21y patients than in controls (P=0.0074). A search for specific CNVs identified two common variants: a 248 kb microduplication on chromosome 6q27, overrepresented in the AO=21y subgroup (5.86%) compared to controls (2.52%, P=0.0079), and a 160 kb microduplication on chromosome 10q11, overrepresented in all BD patients (6.01%) compared to controls (3.67%; P=0.035). Moreover, rare microdeletions and –duplications of a shared 224 kb region on 9q34 were associated with BD (0.6% in patients, 0% in controls; P=0.031). Our data suggest that CNVs have an influence on the development of early-onset (=21 years) BD. Our study adds further support to previous hypotheses that there may be differences in etiology between early-onset and later onset BD patients.

Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early onset extreme obesity in French and German study groups

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Aims: Genome-wide association studies (GWAS) led to the detection of genomic loci involved in obesity and related traits. We aimed to identify novel genes for early onset extreme obesity by a joint approach pertaining to German and French GWAS data sets; additionally we performed a trio-based GWAS on German families.

Methods: We jointly analysed two GWAS with 2,258 individuals of central European origin focussing on extremely obese children and adolescents. Additionally, 424 families with extremely obese young German individuals and both of their parents were analysed by transmission disequilibrium tests. The best hits were followed up in up to 36,734 individuals (of these 8,358 were children and adolescents). For genes adjacent to the detected loci tissue specific expression patterns and regulation of mRNA levels in response to high fat diet feeding were measured by quantitative PCR.

Results: We detected two new loci for (extreme) obesity ($p=6.54\times10^{-8}$ and $p=5.16\times10^{-7}$, respectively) with the later finding being limited to children and adolescents ($p=4.33\times10^{-8}$). The odds ratio for early onset obesity assessed in the replication samples was estimated at ~1.10 per risk allele. Gene expression analysis revealed a striking coregulation of two genes within the new loci suggesting a common biological function.

Conclusions: In our approach including most partners of our NGFNplus network we observed that genetic variants in or near two new obesity genes were robustly associated with early onset extreme obesity. The major polygenes explaining BMI variance overlapped to a substantial degree between children and adults. Functional studies of the implied regions may contribute to new biological insights for the development of obesity and related disorders.

MHC Haplotype Sequencing: An Integrated Approach to Common Disease

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The human major histocompatibility complex (MHC) is recognized as the most important genetic region in relation to common diseases including inflammatory, infectious and autoimmune diseases as well as transplant medicine. Major national and international genome research networks have demonstrated associations between the MHC region (~ 4 MB) and numerous disease phenotypes of interest. To move from the regions of association to the causative variants, the highly complex nature of the MHC needs to be resolved. This requires sequencing individual MHC molecular haplotypes directly and completely, both at the population level and in diseases.

To this aim we have established key resources and technologies: 1) A unique 'Haploid Reference Resource' of 100 human fosmid libraries from a representative German population cohort (PopGen; 200 haploid genomes), and 2) a SOLiD next generation sequencing (NGS) and data analysis pipeline that includes project-specific modules of fosmid detection and sub-genome matching. To generate MHC haplotype sequence information, the following, complementary, approaches have been taken in parallel:
1) Classically, SNP-based mapping and isolation of MHC haplotype-informative fosmids from the clone pools, separate assembly of two MHC fosmid tiling paths, subsequent NGS of those; 2) hybridisation-/PCR-based enrichment of MHC sequences from the fosmid clone pools by use of microarrays coupled with NGS; specifically, HybSelect (Febit), NimbleGen Sequence Capture (Roche), SureSelect RNA baits (Agilent), a microfluidic chip (Fluidigm) and a microdroplet technology (RainDance) are being applied and comparatively evaluated; 3) direct NGS of the haploid clone pools (5000 or 15000 fosmids/pool, respectively). Combination of these approaches allows assembly of first MHC haplotype sequences. Moreover, our lines of research and production provide a valuable platform to tackle highly variable genomic regions and generate haploid sequences on a broader scale.

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Oral presentation abstracts

Symposium II

Genomics of Sporadic Cancer

From genome-wide association studies to whole-genome sequencing in cancer

Ivo Gut

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Technologies for DNA analysis have seen dramatic evolution in the past decades. Genotyping of panels of more than a million SNPs can nowadays be carried out easily and cost-efficiently. Analysing large case-control cohorts with millions of variants allows the identification of disease gene harbouring regions of the genome very economically. We have successfully applied this technology to many different diseases and have managed to identify several genomic regions associated that confer increased susceptibility to different forms of cancer. These identifications give a handle to identify mechanisms that potentially lead to disease. However, they give no indication of the time of onset of the disease. The onset of many forms of cancers is associated with changes of the genome. Cataloguing these acquired variants is the objective of the International Cancer Genome Consortium. We have been carrying out complete genome sequencing of DNA from tumoral and peritumoral tissue to identify somatic variants. The strategies that we have developed to do this will be outlined and first results presented.

AML1-ETO colaborates with the HOX Gene Co-factor MEIS1 in inducing acute leukemia in the mouse bone marrow transplantation model

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AML1-ETO is the most frequent fusion gene in human AML. Previously, we and others have demonstrated that the fusion is not able to cause leukaemia on its own in experimental murine models, but that it needs collaborative partners. However, although mutations such as the FLT3-length mutation and C-KIT mutations were defined as important collaborative genetic events in AML1-ETO positive AML, most human AML1-ETO cases do not carry these mutations, indicating the presence of unkown collaborative partners in these patients. Meis1, a HOX gene co-factor, belonging to the TALE family of homeodomain proteins, has a well established function as a proto-oncogene with a strong collaborative potential in Hox gene associated AML in mice.. First expression analyses confirmed that Meis1 is expressed in patients with AML1-ETO positive AML using Taqman real-time PCR. We then sought to determine if AML1-ETO can collaborate with Meis1 in inducing acute leukemias: single constructs or both genes were co-transfected in 5-FU treated primary murine bone marrow cells by retroviral gene transfer, using MSCV retroviral constructs with an IRES–GFP or YFP cassette. Mice were transplanted with BM cells expressing Meis1 alone (n=10), with BM cells solely expressing the fusion gene (n=10) or EGFP (n=7, control) or with BM expressing both genetic alterations (n=14). None of the mice in the Meis1 and AML1-ETO as well as in the control group developed disease. In contrast, 14 mice transplanted with BM co-expressing AML1-ETO and Meis1 developed lethal disease after a median latency of 102 days. Three mice succumbed to a myeloproliferative syndrome and nine mice died by acute leukemia (6 mice developed AML, 3 mice ALL), which was serially transplantable into secondary recipients (median = 57 days). Immunohistochemistry of various organs of leukemic mice showed massive infiltration with blast cells. In MPS and AML 85 ± 9.3 % of the blast cells co-expressed Gr-1+ and Mac1+. In ALL cases 40 ± 19.9 % of the malignant cells co-expressed Mac1 and the lymphoid-associated B220 antigen. In summary, our data demonstrate for the first time that AML1-ETO can collaborate with Meis1 and identify a novel collaborative partner in t(8;21) positive AML.

Modulation of ERBB receptor-driven cell invasion and proliferation by miR-200c via targeting PLCγ1 and MIG-6 in breast cancer

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The progression of primary to metastasizing tumor cells is a critical process for the survival of breast cancer patients. Unrestrained signaling by overexpression of ERBB receptors results in increased cell motility, metastasis, and proliferation of breast cancer cells. Recently, miR-200c has been shown to regulate epithelial-to-mesenchymal transition (EMT) and invasion by targeting the TGF-ß-induced E-cadherin repressors ZEB1 and SIP1. Here, we aimed at studying a potential involvement of miR-200c also in ERBB receptor-driven cell invasion and proliferation. We first analyzed the effects of miR-200c expression on the ERBB signaling network to quantify the expression and/or phosphorylation states of 20 proteins regulating ERBB-driven cell invasion and proliferation. Combining bioinformatics analysis and whole genome miRNA/mRNA expression profiling with luciferase reporter assays and site-directed mutagenesis, we then identified PLCγ1 and the negative feedback regulator MIG-6 as direct targets of miR-200c. Both proteins have established functions in invasion and proliferation. Furthermore, miR-200c increases cell proliferation by reducing the percentage of cells in G1-phase of the cell cycle, and its overexpression did not sensitize breast cancer cells to the EGFR-targeting molecule gefitinib. We finally analyzed the relative contribution of PLCγ1, MIG-6 and ZEB1 as well as of miR-200c in EGF-induced cell invasion using combinatorial RNAi. The reduction of invasion induced by triple knockdown of the genes was weaker than that of miR-200c overexpression, indicating the potential involvement of yet other targets. Overall, our results suggest that miR-200c expression has a dual tumorigenic function by negatively regulating ERBB-driven cell invasion and positively impacting cell proliferation.

High ALK receptor tyrosine kinase expression prevail ALK mutation as a determining factor of an unfavorable phenotype in primary neuroblastoma

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Purpose: Amplification and activating mutations of the anaplastic lymphoma kinase (ALK)have been postulated to contribute to the pathogenesis of neuroblastoma, the most common extracranial childhood tumor. This study aimed to determine the contribution of genomic ALK alterations and ALK expression levels to the clinical phenotype of neuroblastoma.
Material and Methods: The genomic status and mRNA expression levels of ALK were determined by sequencing, quantitative PCR, and oligonucleotide-microarray analysis in 263 primary neuroblastomas. Allele-specific ALK expression was determined by cloning and sequencing of cDNA fragments. ALK protein expression levels were examined by Western blot analysis. The associations of genomic ALK alterations and ALK expression levels with survival were determined by log-rank test and in Cox regression models.
Results: Amplifications and non-synonymous mutations of ALK were detected in 2/263 and 21/263 neuroblastomas, respectively. Tumors with mutated ALK showed significantly elevated ALK mRNA and protein expression levels, and were associated with unfavorable patients' outcome. Unexpectedly, the wild-type allele was preferentially expressed in most tumors with ALK mutations. In neuroblastomas without ALK mutations, ALK overexpression was strongly associated with prognostic markers of adverse outcome and with poor survival. Clinical courses of patients with ALK mutations and of patients with wild-type ALK showing comparable ALK transcript levels were highly similar. In multivariate analysis, ALK expression but not mutation was an independent factor of adverse outcome.
Conclusion: High expression of ALK prevail ALK mutation as a determining factor of the neuroblastoma clinical phenotype, suggesting that elevated ALK expression in general may represent a specific target for novel therapeutic strategies in neuroblastoma.

National Genome Research Network

Oral presentation abstracts

Symposium III

Animal, Cellular & Tissue Models

Identification of Modifiers of Intestinal Tumor Formation and Progression utilising mouse Chromosome Substitution Strains

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The genetic background of individuals is known to have a major effect on the life-time risk of developing cancer, and on cancer progression. The genetics of cancer susceptibility is however complex (polygenic), and thus it is almost impossible to assess the influence of the individual genetic background of humans on the lifetime risk of developing this disease. Mice share 99% of genes with humans, and also share common diseases. In particular, similar mutations cause colon cancer in mice and humans, and mouse models of human colon carcinogenesis, such as the APC-Min mouse, are available. Differing genetic backgrounds of mouse strains are known to influence tumor multiplicity and the spectrum of organs affected.

We have set up a screen for modifiers of the APC-Min induced tumor phenotype in the mouse intestine using Chromosome Substitution Strains (CSS), mouse strains carrying single chromosomes of the PWD/Ph strain on a C57BL/6 (B6) background. We seek to identify and isolate key modifiers of global epigenetic gene control involved in colon cancer initiation and progression. Massive parallel sequencing of immunoprecipitated methylated DNA (MeDIP-seq) and RNA (RNA-seq) derived from tumor material (mouse and human) is employed to monitor the changes in promoter methylation and gene expression occurring during carcinogenesis, allowing us to enter a new dimension in the genetic analysis of cancer. We expect to isolate multiple genetic traits affecting cancer initiation, progression and recurrence, which will eventually allow us to define low- and high-risk groups among patients, and adapt treatment and follow-up regimes accordingly.

Our first analyses of (B6xCSS) F1 mice carrying the APC-Min mutation have already demonstrated the presence of modifiers of tumor size and multiplicity on PWD/Ph derived chromosomes, proving the suitability of the system for dissecting tumor development. In addition, we show genome-wide analyses of methylated DNA patterns and RNA-seq data.

A hypoxic niche regulates tumor stem cells.

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Glioma growth and recurrence depend on a specialized subpopulation of tumor cells, termed tumor stem cells (TSCs). The molecular mechanisms regulating TSCs are poorly understood. Here, we define a detailed molecular signature of TSCs that is functionally involved in TSC maintenance using a comprehensive transcriptional profiling analysis. Importantly, these signature genes are overexpressed by TSCs in vascular and perinecrotic/hypoxic niches. Specifically, the hypoxic microenvironment plays a key role in the regulation of the TSC phenotype, through the hypoxia-inducible factor (HIF)-2a and subsequent induction of specific TSC signature genes, including ASPHD2, MAML3 and NFATc2. We further demonstrate that these genes regulate TSC maintenance. Consistently, they are overexpressed in newly formed tumors and are linked to a more aggressive clinical behavior. We propose that TSCs are maintained within a hypoxic niche, providing a functional link between the well-established role of hypoxia in stem cell and tumor biology.

The role of the GTPase Arfrp1 for lipid droplet formation and the regulation of lipolysis in adipose tissue

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Aim: ADP-ribosylation factor related protein 1 (ARFRP1) is a monomeric GTPase regulating protein trafficking between intracellular organelles. Due to defective E-cadherin targeting and adhesion defects, conventional Arfrp1-/- embryos die during early gastrulation. In order to define the role of ARFRP1 in the regulation of adipocyte-specific processes we generated and characterized mice with a fat cell-specific deletion of Arfrp1.

Methods: For fat cell-specific deletion of Arfrp1 (Arfrp1ad-/-) we used the Cre/loxP system, generated Arfrp1fox/flox mice and crossed them with transgenic mice (ap2-Cre) expressing the Cre recombinase under the control of an adipocyte-specific promoter (Fabp4/aP2).

Results: Arfrp1ad-/- mice show a severe growth retardation (day 7: 4.9±1.3 vs. 3.5±0.5 g), a significantly reduced body temperature and a reduced survival rate in comparison to control mice (Arfrp1flox/flox). In addition, Arfrp1ad-/- mice are lipodystrophic due to a defective lipid droplet formation in adipose cells. Ratios of mono-, di, and triacylglycerol as well as the fatty acid composition of triglycerides were unaltered. Lipid droplets of brown adipocytes of Arfrp1ad-/- mice were considerably smaller and exhibited ultrastructural alterations such as a disturbed interaction of small lipid loaded particles with the larger droplets, suggesting that ARFRP1 mediates the transfer of newly formed small lipid particles to the large storage droplets. Levels of phosphorylated HSL (hormone sensitive lipase) were elevated and association of ATGL (adipocyte triglyceride lipase) with lipid droplets was enhanced in brown adipose tissue from Arfrp1ad-/- mice. Accordingly, basal lipolysis was increased after knockdown of Arfrp1 in 3T3-L1 adipocytes.

Conclusion: The data indicate that disruption of ARFRP1 prevents the normal enlargement of lipid droplets by inhibiting fusion events, and produces an activation of lipolysis.

Mitochondrial dysfunction and impaired lysosomal degradation due to loss of Parkinson's disease associated protein DJ-1

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Loss of DJ-1 function is a rare cause of autosomal recessively inherited Parkinson's disease. The role of DJ-1 implicates diverse biological functions including chaperone-like functions and antioxidant properties. Although present in various subcellular compartments including cytoplasm and nucleus, targeting of DJ-1 to mitochondria seems to be critical to mediate its physiological cytoprotective role.

Impaired mitochondrial function is critically linked to imbalanced dynamic fusion and fission events of mitochondria, to energetic depression and may subsequently result in the activation of programmed cell. Selective removal of dysfunctional mitochondria by lysosomal degradation pathways is critical for the maintainance of cellular integrity.

We provide evidence that loss of DJ-1 function in MEF causes a prominent disturbance of both, mitochondrial function and morphology, that is linked to decreased basal autophagy and impaired lysosomal degradation.

To determine the role of DJ-1 in PD we used immortalized MEF from a DJ-1 KO mouse model. Mitochondrial and lysosomal function and morphology was studied using FACS-analysis, quantitative morphology, life cell imaging, and ultrastructure analysis.

Loss of DJ-1 was linked to an accumulation of intramitochondrial reactive oxygen species, decreased rates of mitochondrial respiration, a decreased mitochondrial membrane potential, and decrease of mitochondrial branching. Importantly, ultrastructural analyses and lysosomal activity assays revealed disturbed lysosomal degradation pathways, including macroautophagy, in DJ-1 KO cells.

Based on our study of impaired DJ-1 protein function that leads to prominent changes in mitochondrial dynamics and lysosomal degradation, we provide evidence for a novel link between mitochondrial and lysosomal dysfunction in neurodegeneration in PD.

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Oral presentation abstracts

Symposium IV

Systems Biology

Integrative Genomics with a little help from its friend: from genes to function and back

Hans V. Westerhoff and friends

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Originally, the concept 'gene' was closely related to function. With the recognition that parts of DNA molecules serve as carriers of these genes, the concept has become almost synonymous with a piece of DNA. Such a piece of DNA was just transcribed, constitutively, and then translated into a functional protein molecule. The concept has been effective and useful, for a while.
Genomics is inherently different from genetics, as it deals with ensembles of genes/functions that function collectively. Because the minimal size of such collectives exceeds 300, because gene expression occurs on at least 4 levels, and because space and time enter, full genomics is a challenge that exceeds that of bringing a woman to Mars. Genomics therefore needs a little help from its friend, systems biology.

In its top-down mode, Systems Biology has revealed patterns of gene expression, suggesting regulatory modes and molecules involved in the latter. In its bottom-up mode, Systems Biology has helped to understand how some biological functions arise from interactions of molecular components. Success stories in this metabolic dimension include the discoveries of new drug targets, of the possible function of the glycosome, and of the origin of fermentation.

Gene networks have often been treated as one dimensional. Hierarchical regulation analysis now analyzes gene expression in three dimensions, i.e. chemistry (metabolism), gene expression, and time. With respect to transcription regulation of gene expression, we have come across an unexpected mechanism enabling a molecular network to traverse time scales: molecular processes happening at the subsecond time sale can give rise to collective phenomena at the time scale of hours. We shall discuss the example of transcriptional cycling.

Only with this little help from its friend, genomics may begin to deliver on its promise of understanding the molecular basis of the function of entire organisms.

The TREAT1000 project: a step towards an individualisation of medicine

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In many areas of medicine, only small subgroups of patients respond to individual therapy options. This is particularly the case in oncology, where often only a fraction of the patients responds to any of the available therapies. Treatment of non-responders can cause significant side effects, reduces significantly the quality of life and generate high costs to the patient or the health system.

One of the major problems resides in the lack of essential information regarding the molecular characteristics of the tumor at the DNA, RNA, and proteome levels. New sequencing technologies open unprecedented opportunities to investigate acquired genome and transcriptome alterations in the tumor samples in a relatively cost-effective procedure allowing however a data read-out with virtually unlimited dynamic range and high specificity.

We expect, that, in many cases, we will be able to predict therapy response in cancer patients by deep sequencing of the tumor and patient genomes, and the tumor transcriptomes for individual patients, to use this information to establish computer models (‘virtual patients’) able to predict both effects and side effects of different therapies on the individual patient, and to aid in the development of new, more focussed therapies for specifc, genetically defined,s subgroups of patients.

In TREAT1000 (www.treat1000.org), started as a collaboration between the departments of vertebrate genomics and developmental genetics at the MPI-MG, the Comprehensive Cancer Centre and the Department of Pathology at the Charité, and two small companies (Alacris Pharmaceutical (www.alacrispharma.com) and CollabRx (www.collabrx.com)) we propose to use deep sequencing of the genomes and transcriptomes of 1000 oncology patients, to establish predictive models of therapy response, which could aid the oncologists treating these patients to optimise the therapies for these individuals. First results of this program will be described.

A protein-protein interaction network links proteins of cell division pathways to their functional dysregulation in cancer

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Cell division depends on the coordinated interaction of thousands of proteins that comprise of the mitotic spindle, the spindle poles (centrosomes), chromosomal structural proteins and regulatory proteins. Alterations in this network can result in missegregration of chromosomes and genomic instability, altering cellular traits, leading to cancer or cell death.

We have carried out a biochemical analysis of protein complexes from human cell cultures to better understand the molecular events of the regulation and deregulation of cell division in cancer cells. Primary information on protein-protein interactions and protein complex composition is obtained by tandem affinity-purification (TAP) and mass spectrometry. We adapted a high-throughput cloning vector system for stable and inducible expression of bait proteins in isogenic human cell lines. 150 centrosome and signalling components were cloned and characterised as baits for TAP. This approach has defined sofar a network of over 2700 protein-protein interactions (PPIs). These data provided both new information about sub-complexes within the centrosome/spindle and about the interactions of more transiently associated regulatory proteins with these structures.
From the network of 2700 PPIs we selected 20 proteins that were annotated in databases (ONCOMINE, HumanProteinAtlas) to be dysregulated in cancer. Their functional characterisation provided us with 4 novel centrosome proteins whose depletion or overexpression ablates the centrosome/mitotic spindle and leads to aberrant cell division. On a biochemical level we found that these proteins either modulate the activity/expression level of cell cycle regulatory proteins, or control the stability of centrosomal proteins. In addition we confirmed that dysregulation of expression of the identified centrosome components directly correlates with abrogation of spindle or centrosome integrity in tumor tissues.

Genome-wide analysis of protein and mRNA half-lives reveals dynamic properties of mammalian gene expression

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Protein and RNA stability determine dynamic properties of gene expression. However, a systematic comparison of mRNA and protein half-lives has not yet been performed in any organism. Here, we used metabolic pulse labeling with heavy stable isotope encoded amino acids (SILAC) and nucleoside analogs (4-thiouridine) to investigate protein and mRNA turnover in murine fibroblasts. High-resolution mass spectrometry and next generation sequencing allowed us to determine protein and mRNA half-lives for more than 4,000 genes. Intriguingly, while the overall correlation between cellular mRNA and protein abundance is high, half-lives are only marginally correlated. Grouping genes according to their mRNA and protein half-lives revealed functional clusters with similar dynamic properties of gene expression. Our data suggests that knowledge of both mRNA and protein half-lives is crucial to understand regulation of gene expression at the transcriptional and post-transcriptional level.

From Epigenetics to Epigenomics - DNA-methylation patterns along human chromosomes

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In the human DNA-methylation is essential to maintain chromosome stability, for stable gene silencing and to control the developmental potential. Precise mapping of DNA methylation along chromosomes will contribute to the functional understanding of gene regulation and genomic organisation. In the NAME21 project we determined the DNA methylation patterns of all gene promoter regions on chromosome 21 at highest possible resolution using bisulfite sequencing in five human cell types. In parallel we established a high resolution map of the entire chromosome 21 and parts of 20 and 22 using MeDIP analysis on high resolution tiled arrays in three cell types. The comparison of both data sets provides a series of novel insights into the organisation of DNA-methylation along chromosomes and across cell types. Our sequencing data provides one of the most comprehensive data sets on sequence dependent positional information covering 580427 CpG-sites at single chromosome resolution. A detailled analysis of the sequences reveals i) a strongly biased distribution of promoter methylation, ii) a correlation of DNA methylation with GC content but no correlation with the neighboring sequence context, iii) a tendency for helical periodicity of methylation and iv) indications for allele dependent methylation. MeDIP analysis correlates well to sequencing data. MeDIP provides further insights into the chromsomal organisation and cell type specific differences at promoters, inter- and intragenic regions.Together our data shed new light on the nature of DNA-methylation in human cells, the methylome reorganisation in primary and immortalized cells, the connection of CpG patterns to sequence environement and the functional connection to other epigenomic data and to gene regulation. We also discus the impact of the observed genotype-epigenotype correlations for disease related (epi) genetic studies.

Chimera Research in Development Biology - the Ethical Question

Jens Reich

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Human-animal chimeric cells, tissues, embryos and accordingly bred animals are a new powerful tool which is indispensable for present and future research in many branches of human genomics and developmental biology. Experimental research in human beings proper has to observe the strictest ethical boundaries. Therefore animals from several phyla, in particular rodents, serve as model animals. For these the scientific community heeds alsodefinite, but less strict ethical boundaries. This difference is founded in the special ethical value that is ascribed to humans as autonomous and "rational animal" with a moral code of conduct as action guide. Research that by its rational logics suspends the integrity and autonomy of a human being is therefore forbidden, whereas the dignity of experimental animals is to be granted by treating them in a way that does not violate their typal life-style. In practice this means that they must be well kept, must not be tortured, and that experimental projects cannot and need not obtain their informed consent.

Both branches, human bioethics and animal bioethics present a number of potential ethical conflicts to which the scientific community must take a humanistic position and has to contribute to the public discourse. One traditional attitude, namely that the scientist obeys the scientific conduct of behaviour within his laboratory and is not responsible for what is being done outside with his results, is no longer tenable. Fundamental research and its application are too much intertwined already at their start that splendid isolation and neat separation is no longer an option.

With chimeric research, in particular if involving human "sources" and "products", a new chapter of this dialogue between science and society has been opened. It is tantalizing for several reasons. At first the categories of human ethics and animal protection loose their traditional enclosures. And second, we human beings have a deeply rooted, mythically fortified aversion against, even fear of, blends between species, in particular if humans are included. We scientists must therefore say very clearly, in public, what we are doing or planning, why we act as we do, what boundaries we observe as self-imposed conduct of decent behaviour, and which strict regulations we are prepared to accept.

The lecture will explain, what chimera and hybrids are, will present actual examples of chimeric research, and will explain rules of proper action as the speaker as a person sees it.

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Oral presentation abstracts

Symposium V

New Technologies

Protein folding and misfolding: a major challenge for modern disease research

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Protein misfolding causes several sporadic and genetic diseases, including neurodegenerative illnesses such as Alzheimer's, Parkinson's or Huntington's disease. Protein misfolding diseases are characterized by the accumulation of protein aggregates, which have detrimental consequences for the cellular system. An increasing body of evidence indicates that small, soluble amyloid oligomers, rather than large, mature amyloid fibrils, are the major toxic species that cause dysfunction and cellular toxicity. An exact characterization of the pathogenic molecules and the cellular processes they are involved in, however, is still missing.

We have used cell-free, cell-based and *in vivo* model systems to identify and characterise small molecules and proteins that modulate the amyloidogenesis of neurodegenerative disease proteins such as huntingtin, α-synuclein or amyloid-β. Both accelerators and inhibitors of protein misfolding were identified. Recently, e.g., the polyphenol EGCG was discovered to bind directly to natively α -synuclein or amyloid- β and to efficiently redirect amyloid assembly. Novel modulators of protein misfolding pathways were also predicted using a bioinformatic data integration strategy. A brain-specific protein-protein interaction (PPI) network for Huntington's disease (HD) was created, linking 14 potentially dysregulated proteins directly or indirectly to the disease protein. One of the proteins identified in the network was the neuron-specific CRMP1, which we predicted to be abnormally down-regulated in HD pathogenesis. In a *Drosophila* model of HD, overexpression of CRMP1 suppressed polyQ-mediated Htt aggregation and improved photoreceptor degeneration and motor impairment. We demonstrated that interaction partners have a protective effect on Htt folding and the disease phenotype. We propose that this approach is applicable to a wide range of other diseases. Proteins that interfere with protein misfolding pathways might be targets for future therapy development.

Structural Variations in the Genome –Using Next-Generation Sequencing to obtain Insights into their Origin, Extent, and Functional Impact

Jan Korbel

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In my presentation I will highlight the current research of my group at EMBL, which combines large-scale experimental technologies and computational data mining approaches for studying the genomic extent and the mutational formation mechanisms of genomic structural variants (SVs) in humans. SVs, frequently referred to as copy-number variants, are >1kb deletions, duplications, insertions, and inversions responsible for most genetic variation in the human genome. We recently developed high-resolution and massive paired-end mapping, an approach that involves ultrafast sequencing of the end-stretches of 3kb genomic DNA fragments and mapping them against a reference genome to identify SVs at subkilobase resolution. Applying this approach to two human genomes allowed us to obtain insights into the mutational origins of SVs and into the extent at which SVs affect human genes. This session will also explore one aim of the “1000 Genomes Project”, which is the generation of a global map of rare and common SVs in humans, that EMBL is developing analysis approaches for. Furthermore, these approaches are expected to help facilitate, in the near future, the design of next-generation sequencing-based disease association studies that may, for instance, help elucidate the functional impact of chromosomal aberrations associated with human diseases, including cancer.

Cell Array-Based Functional Analysis of Human Chromosome 21 Gene Promoters

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Given the inherent limitations of in silico studies relying solely on DNA sequence analysis, the functional characterization of mammalian promoters and associated elements requires experimental validations through the cloning and analysis of putative promoter regions. Focusing on systematic promoter studies for human chromosome 21 (HSA21), we cloned 182 promoters of 2,500 base pairs in length and proceeded to reporter gene assays on transfected cell arrays. Of those, 56 promoters were found active in HEK293 cells; these data correlate well with the corresponding endogenous transcriptional activity measured by RNA-seq and with the promoter occupancy pattern of RNA polymerase II obtained by ChIP-seq.

Besides, an additional set of 51 promoters could be activated by treatment of the cells with Trichostatin A (TSA) or depletion of fetal calf serum, showing that these promoters also have the capacity to drive transcription. TSA treatment preferentially activated promoters containing CpG islands, indicating that promoter-reporter constructs are sensible to endogenous DNA methylation-mediated silencing. We tested a subset of 62 truncated HSA21 gene promoters (~500 bp) and found that proximal promoter regions were generally sufficient to drive gene expression. However, data indicates that 1/3 of the promoters contain positive or negative cis-acting elements in distal promoter regions, as determined from response patterns to TSA treatment and serum depletion.

The global analysis of HSA21 gene promoters presented here shows that promoter reporter activities largely recapitulate endogenous expression states. In addition, data allow for classification of promoters according to (1) the promoter length sufficient for reporter gene expression, (2) responses of promoters to external stimuli, and (3) the presence of cis-acting response elements within distal promoter regions. These data provide the first insight into promoter functions on the scale of a whole chromosome.

Prioritizing Disease Genes from Association Studies using Functional Similarity

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Genome-wide association studies frequently result in hundreds of candidate genes. Therefore, computational prioritization methods are required for identifying the most promising candidates for further experimental validation. Here, we describe MedSim, a new approach for prioritizing candidate genes and proteins using automatically derived functional profiles based on the Gene Ontology. Candidates are ranked according to the computed similarity between their functional profiles and the profile of the disease of interest. We validated our method using leave-one-out cross-validation and artificial quantitative trait loci. MedSim achieved an excellent performance with an AUC of up to 0.90 and a sensitivity of over 70 %.

The implementation of the MedSim method is publicly available via our FunSimMat web server: http://www.funsimmat.de

TransgeneOmics: a platform for systems wide protein function analysis in metazoans

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Both normal and pathological phenotypes at any biological level are result of highly interconnected networks of interacting components. Understanding this complexity would place the know disease genes into molecular pathways, providing a way towards novel treatments. Although we can now determine genome sequence with remarkable ease and accuracy the identity, levels and patterns of localization of the encoded protein products cannot be accurately predicted from the genome sequence.
We have previously developed methods for analysis of protein function in metazoans under endogenous expression control using generic tag based localization and purification assays. Extending this approach to systems scale would drastically improve our ability to assign molecular functions to previously unstudied proteins and will reveal new players even in well-understood functional processes relevant to human biology and disease pathways. To address this problem have established an efficient and scaleable technology platform including an efficient transgenic pipeline, reliable multipurpose tags and sensitive, high-resolution methods for data acquisition.
We are currently developing genomics scale transgenic resources in several metazoan models, which would permit the localization and biochemical purification of any protein of interest.

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Oral presentation abstracts

Symposium VI

Transfer from Genomics to Application

Virtual and dynamic small molecule screening in academic drug discovery

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Small molecule inhibitors of proteins are valuable chemical tools for the functional characterization of proteins and can serve as starting points for drug development. Over the recent years we have established an infrastructure for academic drug discovery and the paper will discuss method development and contributions in this field on the basis of case studies.[1-4]

Fragment-based methods in drug discovery have become popular during recent years as they can provide protein ligands with significantly increased ligand efficiency.[1]

In cases with a well-defined binding pocket as found in the protein tyrosine phosphatase Shp2 virtual screening of chemical libraries can provide valuable starting points for protein ligand development.[2]

If, however, limited structural information is available for the protein or if the protein surface is adaptive, template-assisted search strategies can be superior. We have introduced Dynamic Ligation Screening (DLS) as a method for the discovery and iterative development of small molecule fragments binding to defined protein sites.[3] DLS enables the rapid and site-directed identification of low-affinity binders by exploiting template-assisted fragment assembly. In DLS the effects of single fragments on a ligation reaction are recorded via a biochemical assay employing one fragment per microtiter plate well. Enzymatic assays as well as protein binding assays can be adapted to the DLS method. Currently we aim at extending DLS from reversible reactions to irreversible ligation reactions.[4]

References: [1] M.F. Schmidt, J. Rademann, *Trends Biotechnol.* 2009, *27*, 512-521. [2] K. Hellmuth, S. Grosskopf, C. T. Lum, M. Würtele, N. Röder, J.P. von Kries, M. Rosario, J. Rademann, W. Birchmeier, *Proc. Nat. Acad. Sci. USA* 2008, 7275-7280. [3] M.F. Schmidt, A. Isidro-Llobet, A. El-Dahshan, M. Lisurek, R. Hilgenfeld, J. Rademann, *Angew. Chem. Int. Ed.* 2008, *47*, 3275-3278. M.F. Schmidt, A. El-Dahshan, S. Keller, J. Rademann, *Angew. Chem. Int. Ed.* 2009, *48*, 6346-6349. [4] Ahsanullah, P. Schmieder, R. Kühne, J. Rademann, *Angew. Chem. Int. Ed.* 2009, *48*, 5042-5045.

Specific miRNA fingerprints in patients' blood samples as novel diagnostic tool

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For many human diseases, including cancer, there is still a lack of high-performing biomarkers in clinical practice. The role of microRNAs in pathogenesis and the power to associate expression changes with disease states underscores their value as such molecular biomarkers. The expression of miRNAs has demonstrated to be highly specific for tissues and developmental stages and has also been proven to allow for molecular classification of tumors. miRNAs are not only found in tissues but also in human blood, both in mononuclear blood cells and as free circulating nucleic acids, highlighting their potential as stable blood-based markers for the detection of cancer or other human diseases.
This proof of principle investigated whether there is a sufficient number of miRNAs deregulated in blood cells of lung cancer patients to be able to distinguish between cancer patients and controls. Using the fully automated Geniom Real Time Analyzer platform, the miRNA biomarker expression in 17 blood cell samples of patients with non-small cell lung carcinomas (NSCLC) and in 19 blood samples of healthy controls were analyzed. 27 miRNAs have been found to be significantly deregulated in blood cells of lung cancer patients as compared to the controls. Moreover, using a subset of 24 miRNA biomarkers allowed for discriminating between blood cell samples of tumor patients and controls with an accuracy of 95.4% [94.9%-95.9%], a speci?city of 98.1% [97.3%-98.8%], and a sensitivity of 92.5% [91.8%-92.5%]. These findings support the idea that neoplasia leads to a deregulation of miRNA expression in blood cells of cancer patients compared to blood cells of healthy individuals, and that miRNA biomarker signatures can be used for blood-based diagnosis of human diseases such as cancers. Moreover, the high reproducibility and throughput enables that this technique can be easily transferred into clinical practice.

Human host cell factors crucial for influenza virus replication identified by genome-wide RNAi screen

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With a propensity to cause seasonal epidemics and reoccuring pandemics, the influenza virus represents a global threat to public health. High mutation rates facilitate the generation of viral escape mutants rendering vaccines and drugs directed against virus-encoded targets ineffective. In contrast, targeting host cell determinants temporarily dispensable for the host but crucial during acute virus replication could prevent viral escape. Here we report the identification of 287 human host cell genes influencing influenza A virus replication using a genome-wide RNAi screen. Subsequent in-depth investigation of a subset of these factors (known and novel) revealed their life-cycle stage relevance and intriguing mechanistic insights. We also show that a small molecule inhibitor of one of our hits reduces influenza virus replication by more than two orders of magnitude, an effect accompanied by impaired splicing of the viral M2 mRNA. Importantly, the majority of factors tested were essential for replication of both the highly pathogenic avian H5N1 and the current pandemic swine-origin H1N1influenza viruses, indicating a broad dependency of variant influenza viruses on these factors. Thus, these results highlight the applicability of our genome-wide RNAi approach not only for the dissection of virus-host interactions but also for the identification of potentially broad-spectrum antiviral targets.

Integration of genomic and proteomic data to unwire chemoresistance mechanisms

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Critical for the efficacy of anti-cancer therapies is the identification of cellular processes that are deregulated in individual tumors. However, efficiently blocking a single deregulated protein is mostly not sufficient to block tumor growth demonstrating the complexity of the underlying signaling networks. Additional efforts are necessary to gain insights into the molecular basis of cancer progression and drug resistance.

Recent large-scale sequencing efforts revealed that genomic aberrations of human cancers converge on the activation of a limited repertoire of signaling pathways and thus reduce the number of cancer-relevant target proteins. The large-scale analysis of such proteins and their activation states is an obvious strategy in drug discovery research, and reverse phase protein microarrays (RPPA) were chosen for a highly sensitive and quantitative analysis. RPPAs permit the parallel analysis of the abundance and activation state of many individual target proteins in hundreds of different samples. We have applied RPPA for the quantitative profiling of 84 non small cell lung cancer (NSCLC) cell lines and integrated additional information available from mutational analyses, gene amplification studies, as well as EC50 data for tumor-targeting drugs. RPPA-based profiling correctly identified cell lines with amplified EGFR or ERBB2 and detected quantitative differences in the activation state of other cancer-relevant pathways, for example, signaling through PTEN, AKT, as well as p53. Early events of tumorigenesis, for example K-RAS mutations, did not align with clusters of downstream signaling pathways. However, low EC50 values for anti-EGFR targeting correlated well with cell lines overexpressing EGFR or HER2. Thus, RPPA-based proteome profiling will certainly lead to a more comprehensive understanding of cancer-relevant signaling networks and presents a useful platform to integrate information from different experimental approaches.

Genome-wide analysis of deregulated microRNAs in prostate cancer

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Prostate cancer is the most frequent solid cancer in men and the second most frequent cancer-related death cause in western countries. The accumulation of genetic alterations during the development and progression of prostate cancer is very complex and leads to changes in gene, microRNA and protein expression. One aim of the consortium IG Prostate Cancer is to get insights into the broad molecular changes in tumors by analyzing different molecular levels in the same patient cohort. We extracted genomic DNA, total RNA including microRNA and protein from 50 high-grade tumors and 50 benign prostate tissue samples. The objective of the present subproject was to analyze the level of 667 different microRNAs by using quantitative real-time PCR-based arrays. Concordance between protocols with and without preamplification of the microRNA pool was very high. Compared to previously published microarray studies, the sensitivity and dynamic range of microRNA detection were clearly enhanced. microRNA Ct values were normalized using quantile normalization followed by modified t-Test to extract variable miRNAs between clinical subgroups. We identified 161 microRNAs differentially expressed (p-value<0.05; at least two-fold change) between tumor and benign prostate tissues, and several microRNA associated with tumor progression. Selected microRNAs were inhibited or enhanced in prostate cancer cell lines PC3 and LNCaP to screen for changes in proliferation, migration and apoptosis. The microRNA abundance in tissues was also compared to the level in serum samples in order to select potential predictive surrogate biomarkers. Furthermore, we integrate microRNA- and gene expression data of the same patient cohort to highlight potential target genes and downstream pathways. In summary, microRNA expression data was explored for their relevance in prostate cancer and can be integrated into broad molecular analysis to highlight interactions with genomic, transcriptomic and proteomic data.

Risk prediction of myocardial infarction using a weighted genetic score--the German MI Family Study

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AIM: Recent genome wide association studies discovered several susceptibility genes for myocardial infarction (MI). Individually, these genetic variants confer only a modest risk increase. The goal of the present study was to determine if a score build on the 10 currently known risk alleles with genome-wide significance for MI leads to better risk prediction and discrimination of cases and controls

METHODS: We constructed an additive genetic score using single-nucleotide polymorphisms (SNPs) from 10 MI susceptibility genes, weighting each SNP with its reported effect. We tested this score by regression analysis in three independent case-control samples from the German MI Family study comprising a total number of 3435 controls and 4488 cases.

RESULTS: In each of the three case-control cohorts the weighted score derived from 10 MI risk genes was significantly associated with MI risk with odds ratios strongly exceeding the risk conferred by individual SNPs (GerMIFs I OR 3.9 [2.8-5.4], p = 6.23E-16; GerMIFs II OR 3.7 [2.6-5.1], p =4.42E-15; GerMIFs III (KORA) OR 2.4 [1.8-3.2], p =2.77E-09).

CONCLUSIONS/INTERPRETATION: In our three case-control samples the combination of risk alleles from 10 recently identified MI loci shows a strong additive effect that was consistently replicated. However, on an individual level the discrimination of cases and controls through the genotype score remains difficult. In the near future the addition of further newly identified MI risk loci into the score could improve individual risk prediction and might eventually lead to clinical application of a genetic risk score for MI.

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Symposium I

Genomics of Common Disease

Coordinates in the universe of node negative breast cancer revisited

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We present a global picture of the natural history of node-negative breast cancer in which two of three important biological processes have outstanding prognostic consequences. We propose that the transition from slow to fast proliferation of the tumor leads to the most dramatic aggravation of prognosis. Secondly, immune cell infiltration is of major importance to prevent disease progression in fast proliferating breast carcinomas, regardless of estrogen receptor status. In the absence of endocrine treatment, steroid hormone receptor expression as a third axis is of limited prognostic importance. Dissecting tumors according to these three major biological axes will allow further understanding of biological processes relevant for tumor progression in node-negative breast cancer patients.

ERBB2 induces an anti-apoptotic expression pattern of Bcl-2 family members in node negative breast cancer

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Purpose: Members of the Bcl-2 family act as master regulators of mitochondrial homeostasis and apoptosis. We analyzed whether ERBB2 influences prognosis of breast cancer by influencing the pro- versus anti-apoptotic balance of Bcl-2 family members. Experimental Design: ERBB2 regulated Bcl-2 family members were identified by inducible expression of ERBB2 in MCF-7 breast cancer cells and by correlation anaysis with ERBB2 expression in breast carcinomas. The prognostic relevance of ERBB2-regulated and all additional Bcl-2 family members was determined in 788 untreated node-negative breast cancer patients. The biologic relevance of ERBB2-induced inhibition of apoptosis was validated in a murine tumor model allowing conditional ERBB2 expression. Results: ERBB2 caused an anti-apoptotic phenotype by upregulation of MCL1, TEGT, BAG1, BNIP1 and BECN1 as well as downregulation of BAX, BMF, BNIPL, CLU and BCL2L13. Upregulation of the anti-apoptotic MCL1 (P=0.001, HR=1.5) and BNIP3 (P=0.024, HR=1.4) was associated with worse prognosis considering metastasis-free interval, whereas clusterin (CLU) (P=0.008, HR=0.88) and the pro-apoptotic BCL2L13 (P=0.019, HR=0.45) were associated with better prognosis. This indicates that ERBB2 alters expression of Bcl-2 family members in a way that leads to adverse prognosis. Analysis of apoptosis and tumor remission in a murine tumor model confirmed that the prototypic Bcl-2 family member Bcl-xL can partially substitute for ERBB2 to antagonize tumor remission. Conclusion: Our results support the concept that ERBB2 influences expression of Bcl-2 family members to induce an anti-apoptotic phenotype. Antagonization of anti-apoptotic Bcl-2 family members may improve breast cancer therapy, whereby MCL1 and BNIP3 represent promising targets.

Epigenetic silencing of a Lim homeobox transcription factor LHX9 modulates the invasive behaviour of malignant gliomas of childhood

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High-grade gliomas (anaplastic astrocytoma and glioblastoma) of childhood represent approximately 7 % of paediatric brain tumors. They are highly invasive tumors and respond poorly to conventional treatments in contrast to pilocytic astrocytomas, which usually are well demarcated and frequently can be cured by surgery. The molecular events for this clinical relevant finding are only partially understood. To identify aberrantly methylated genes that may be involved in the tumorigenesis of pediatric high-grade gliomas, we performed a microarray-based differential methylation hybridization (DMH) approach and found frequent hypermethylation of the LHX9 (human Lim-homebox 9) gene encoding a transcription factor involved in brain development. Bisulfite genomic sequencing and combined bisulfite restriction analysis (COBRA) showed that high-grade gliomas were frequently methylated at two CpG-rich LHX9 regions in comparison to pilocytic astrocytomas and normal brain tissues. The LHX9 hypermethylation was associated with suppressed mRNA expression in pediatric high-grade glioma samples and corresponding cell lines. This epigenetic modification was reversible by pharmacological inhibition (5-aza-2' -deoxycytidine) and reexpression of LHX9 transcript was induced in pediatric glioma cell lines. Exogenous expression of LHX9 specifically inhibited glioma cell migration and invasion in vitro without directly affecting cell proliferation and apoptosis, suggesting possible implication of LHX9 in the migratory phenotype of high-grade gliomas.
Our results demonstrate that the frequent epigenetic silencing of LHX9 gene is involved in the migratory and invasive potential of pediatric malignant gliomas

flowDeconvolutor: A reproducible and flexible approach to flow cytometric cell cycle analysis

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The regulation of cell cycle is fundamental for all organisms and a large portion of related studies is performed with flow cytometry, where the analysis of DNA content histograms has proven its power in investigating impact of inferences (siRNA, chemical compounds, miRNA mimics, etc) or disease (i.e. breast cancer) on cell cycle. Despite of the high-throughput and rich information, most data harvested from the flow cytometry has been subjectively analyzed in an over-simplified way partly due to the lack of low-cost and efficient analytical tools. We introduce DNAdeconvolutor, an open-source Bioconductor package that allows users to analyze DNA content histograms derived from flow cytometry. The package performs reproducible deconvolution of DNA histograms and reports statistics of cell cycle phases. A novel working curve mode enables flexible calibration of correlation between the results of package and those of independent methods (e.g. BrdU S-phase specific staining). Both descriptive and inferential statistical tools are shipped along with the package to allow the analysis of cell cycle with the power of statistics. Implementation of the graphical user interface (GUI) and an one-step end-to-end function paves a flat learning curve to proficient analysis, while the package also supports advanced flexible analysis in a wide range of working conditions by allowing user to adjust a rich panel of parameters.
The DNAdeconvolutor package is released under the LGPL license and is freely available at http://www.bioconductor.org.

A GLUT1 mutation in patients with spastic paraplegia and paroxysmal dyskinesia

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Glut1 is the glucose transporter of the blood brain barrier, thus a crucial molecule to deliver the most important energy carrier to the brain. Mutations in Glut1 have been found in Glut1-deficiency syndrome, a severe syndrome of early childhood with drug resistant epilepsy, microcephaly and progressive mental retardation. Recently, we detected mutations in Glut1 in patients with paroxysmal exercise-induced dyskinesia. Previously, Auburger and colleagues described a family with paroxysmal dyskinesia in combination with mild gait ataxia and constant spastic paraplegia in some patients (CSE, DYT9; Genomics 1996; 31:90-94). The family included 18 affected family members and 11 unaffected probands. Linkage had been found close to the Glut1 locus on chromosome 1p. We now re-analyzed the family clinically, recruited more family members, and sequenced the Glut1 gene SLC2A1. We found a p.R212C missense mutation. Functional analysis of the mutation expressed in Xenopus laevis oocytes revealed a significant reduction of glucose uptake compared to the wildtype. Western blots indicated a possibly slight reduction in protein stability, but surface expression was not altered in oocytes. Our findings indicate that constant spastic paraplegia may be part of the phenotype associated with Glut1 mutations, which appear to be more common than previously assumed. Since such patients can be treated successfully with a ketogenic diet, mutation screening should be performed in appropriate cases.

Recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 predispose to idiopathic generalised epilepsies

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Idiopathic generalised epilepsy (IGE) accounts for 30% of all epilepsies. Despite a predominant genetic aetiology, the genetic factors predisposing to IGE remain elusive. Studies of structural genomic variations have revealed a significant excess of recurrent microdeletions at 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11, and 22q11.2 in various neuropsychiatric disorders. This study investigated the impact of these six microdeletions on the genetic risk to common IGE syndromes. The candidate microdeletions were assessed by high-density single nucleotide polymorphism arrays in 1234 IGE patients from North-Western Europe and 3022 German population controls. Microdeletions were validated by quantitative PCR and their breakpoints were refined by array comparative genomic hybridisation. In total, 31 IGE patients (2.5%) carried one of the six microdeletions compared to nine controls (0.3%) (OR=8.6, 95% CI: 4.1–18.2, P=1.1x10-11). Microdeletions were observed at 1q21.1 (IGE/contr.: 1/1), 15q11.2 (IGE/contr.: 12/6), 16p11.2 (IGE/contr.: 1/0), 15q13.3 (IGE/contr.: 9/0), 16p13.11 (IGE/contr.: 6/2), and 22q11.2 (IGE/contr.: 2/0). Significant associations with IGE were found for the microdeletions at 15q11.2 (OR=4.9, 95% CI: 1.8–13.2, P=4.2x10-4), 15q13.3 (Fisher's exact test, P=1.4x10-5), and 16p13.11 (OR=7.4, 95% CI: 1.3–74.7, P=0.009). Parental transmission could be examined in 14 families. While ten microdeletions were inherited, four microdeletions occurred de novo at 15q13.3 (n=1), 16p13.11 (n=2), and 22q11.2 (n=1). Eight of the transmitting parents were clinically unaffected, suggesting that the microdeletion itself is not sufficient to cause epilepsy. The present results indicate an involvement of microdeletions at 15q11.2, 15q13.3 and 16p13.11 in epileptogenesis. Although the microdeletions investigated are individually rare (<1%) in IGE patients, they collectively account for a significant fraction of the genetic variance of common IGE syndromes.

Biophysical characterization of a T-type Ca channel mutation and an HCN4 polymorphism found in a family with idiopathic generalized epilepsy

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T-type Ca2+ channels regulate neuronal firing by initiating low-threshold spikes that can generate bursts of action potentials. Previous studies have shown that genetic variations in the T-type Ca2+ channels associate with childhood absence epilepsy (CAE). We identified a mutation in the CACNA1H gene in a family with idiopathic generalized epilepsy, predicting the G1158S substitution located in the cytoplasmic loop connecting domains 2 and 3 of the channel. Furthermore, one affected and one non-affected member of this family also had a polymorphism in the HCN4 channel, P1117L. Both genetic variations were not found in 253 ethnically matched controls. To examine the potential epileptogenic effect of the detected variations, we compared the electrophysiological behavior of mutant and wild type (WT) channels: T-type Ca channel currents were recorded from the transfected tsA 201 cells, whereas the HCN4 channels were expressed in Xenopus laevis oocytes and their currents analysed using automated two-voltage clamp. The G1158S mutation caused small but significant gain-of-function changes: a faster recovery from inactivation and a depolarizing shift of steady-state inactivation which could result in an increased susceptibility for neuronal bursts. The P1117L HCN4 polymorphism caused a significant loss-of-function of these channels by reducing peak tail current amplitudes, which fits nicely with the seizure development in the HCN2 knock-out mice. Although the variation does not completely co-segragate with epilepsy in this family, the observed functional defect together with the fact that the variation was not found in controls, may implicate it as a relevant factor in seizure development. Moreover, both T-type Ca channels and HCN4 channels are highly expressed in thalamocortical loops and thus might be involved in the generation of seizures in idiopathic generalized epilepsy.

Allelic promoter variants accumulated in epileptic patients: indication for mRNA-expression alterations of corresponding genes

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Episodic central nervous system diseases including temporal lobe epilepsy (TLE) represent frequent neurological disorders. In TLE, a main clinical problem is given by the development of pharmacoresistance. Many patients with pharmacoresistant TLE, therefore, undergo surgical treatment with hippocampal removal, frequently resulting in seizure control. Hippocampal biopsies from epilepsy surgery (n>150) provide the unique opportunity to correspondingly analyze allelic variants and gene expression in human brain tissue. Using PCR and quantitative real-time RT-PCR systems, we have addressed whether promoter-associated allelic variants present in epileptic and migraine patient collectives alter corresponding gene expression. These analyses revealed a significantly increased expression of the succinic semialdehyde dehydrogenase- (SSADH) gene in individuals, that carried an SNP-variant of SSADH associated with idiopathic generalized epilepsy. Furthermore, we observed increased mRNA expression of the 5-HT1A (5-HT-serotonin) receptor in individuals harboring the GG-genotype of the 5-HT1A receptor promoter polymorphism that is associated with avoidance of physical activity during a migraine attack. These analyses provide a first insight into alterations in human hippocampal gene expression caused by allelic variants associated with episodic brain disorders. Our current experiments concentrate on (a) in vitro approaches to understand the functional significance of promoter regulation of the above mentioned allelic variants and (b) large scale analysis of DNA/mRNA from human hippocampal specimens using whole genome approaches. Results of these ongoing experiments will be presented as well.

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Genetic basis of Levetiracetam pharmacoresistance and side effects in human epilepsy

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Pharmacoresistance and substantial side effects represent serious problems in the treatment of epilepsy patients with anti-epileptic drugs (AEDs). To date, the molecular basis of drug resistance in epilepsy remains elusive. Only recently, with the identification of the synaptic vesicle protein SV2A as high-affinity binding site for the AED Levetiracetam (LEV), the presynapse has come into focus as a target for AEDs. However, it is still unknown how LEV affects the function of SV2A. LEV is an effective AED in many epilepsy patients refractory to other AEDs. Intriguingly, 90% of all LEV-treated patients can be divided into two distinct groups: “a-priori non-responders” versus “primary responders”. In this study, we have classified 503 patients according to their initial response to LEV. We have then sequenced the SV2A gene and its promoter region using genomic DNA from blood of these patients in order to identify genomic variants associated with an a-priori non-response. As a subgroup of these patients underwent surgical treatment to alleviate the seizure phenotype we analyzed the resulting hippocampal biopsy specimens with regard to SV2A expression levels by quantitative real time RT-PCR and immunofluorescence and microscopy. We found that SV2A expression was significantly reduced in the a-priori non-responder group. We are furthermore analyzing expression of other components of the presynaptic release machinery in these samples to gain insight into the mechanism underlying the anti-epileptic action of LEV.

Genome-wide association study in migraine without aura

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Background. Migraine is a common primary headache disorder, characterized by attacks of severe, throbbing headache and autonomic nervous system dysfunction. The majority of patients suffer from migraine without aura (MO), i.e. their headaches are not preceded by neurological disturbances. Epidemiological studies have provided evidence for an important genetic component in migraine. However - in contrast to familial hemiplegic migraine, which is caused by mutations in ion channel / transporter genes - no genetic variants associated with the common types of migraine have been identified.
Goals. To identify common variants associated with MO.
Patients. A cohort of 838 clinic-based German MO patients was recruited at the Department of Neurology of Munich University. All patients were subjected to an extensive previously validated headache questionnaire. For diagnosis of MO, the criteria of the International Headache Society were used. In addition, a population-based cohort Australian MO cohort was available, comprising 764 unrelated cases. Population-matched controls were recruited from existing GWA studies: for the German part, 840 controls were obtained from the KORA study; for the Australian part, 3772 unrelated controls were available.
Genetic analysis. Genotyping was performed using the Human610-Quad v1 array (Illumina). For the German cases, mean call rate was 99.40%.
Preliminary results. Association analysis was performed using logistic regression (allelic/additive model). Separate analysis of both samples was without significant results; by contrast, joint analysis yielded one SNP with genome-wide significance ($p<10e-8$).
Future perspectives. For replication, the identified variant will be analysed in two further Caucasian MO samples; in case of positive results, functional analyses will be performed. In addition, in silico replication of the top MO finding will be performed in a large MA sample, available through the International Migraine Genetics Consortium.

High-density SNP array linkage and CNV analyses in familial forms of common migraine

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Migraine is a prevalent primary headache disorder with a high heritability but a poorly understood genetic basis. With the long-term goal of identifying genes underlying common forms of migraine, we have collected a large number of multiplex migraine families, parent-child trios and single patients of German origin, enriched for migraine with aura (MA), and started genetic mapping studies. The initial family sample contains 380 individuals in 41 families, with 3-12 affected individuals available for genotyping in each family. We used Affymetrix 6.0 arrays for genomewide high-density genotyping which allows us not only to perform linkage analysis with SNP markers but also to detect copy number variations (CNVs) associated with migraine. In the large multiplex families, we did not detect linkage to any of the three known genes involved in familial hemiplegic migraine (FHM), confirming that FHM genes are not responsible for common migraine in most families. Our preliminary results have identified a single MA locus in one family which overlaps a previously reported MA locus, and next-generation sequencing of this genomic region is underway to uncover the causative MA mutation. Moreover, we identified candidate linkage regions in most of the other families, some of which are < 3 Mb and would have been missed by microsatellite-based linkage scans. Our ongoing sequencing efforts concentrate on functional candidate genes mapping to overlapping regions putatively linked in two or more families and on strong functional candidate genes like ion-channel genes or genes involved in neurotransmitter metabolism and cortical excitability. In addition, we perform CNV analyses and family-based association studies, which are complemented by genome-wide association studies within an international consortium. Our results may ultimately help to better understand the complex genetic architecture of common forms of migraine.

GENOME-WIDE ASSOCIATION MAPPING OF SUSCEPTIBILITY ALLELES PREDISPOSING TO IDIOPATHIC GENERALISED EPILEPSY

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Idiopathic generalised epilepsies (IGEs) are genetically determined (heritability: 80%) and presumably display a polygenic predisposition in the majority of affected individuals. Up to date, the genetic variants predisposing to common IGE syndromes remain elusive. To search for common genetic risk factors, we have set up a two-staged genome-wide association (GWA) study on common IGE syndromes, using the Affymetrix Genome-Wide Human SNP Array 6.0. The Stage-1 discovery sample comprised 1216 IGE patients of Western-European ancestry and 2883 German population controls (PopGen & KORA cohorts). Association analysis was conducted for the entire IGE sample and three subsamples, comprising 758 female IGE patients (IGE-fem), 581 IGE probands affected by idiopathic absence epilepsy (IAE) and 474 probands affected by juvenile myoclonic epilepsy (JME). The GWA results revealed a promising number of genome-wide significant associations, in particular in the IGE-fem subsample. The Armitage Trend Test (ATT) showed 44 association hints achieving a significance threshold of ATTp<10e-7 in the four IGE samples (IGE: n=11; IGE-fem: n=17; IAE: n=11; JME: n=5). In the IGE-fem subsample nine out of 17 top-ranked SNPs (ATTp<10e-7) were observed in a single segment spanning 800kb on chromosome 10p11. This finding reinforces clinical evidence for gender-related genetic risk factors. The observation of mainly distinct association signals in the IAE- und JME subsamples suggests that different configurations of genetic risk factors may predispose to absence and myoclonic seizures. We are currently evaluating 133 Stage-1 association hints (ATTp<10e-5) in a Stage-2 confirmation sample including 932 European IGE patients and 1116 ethnically matched controls. The comprehensive survey of the most relevant genomic risk factors will enhance prospects to specify individual risk profiles and to differentiate their leading epileptogenic pathways.

Interference with AML1/ETO leukemogenic function by peptides targeting the oligomerization domain

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AML1/ETO is generated by the chromosomal translocation t(8;21) which appears in about 12% of all de novo acute myeloid leukemias. It consists of the DNA-binding domain of the hematopoietic transcription factor AML1 and almost the entire ETO protein which functions mainly as a transcriptional repressor. Essential for AML1/ETO function is the oligomerization domain of the chimeric protein. The alpha-helical nervy homology region 2 (NHR2) within ETO mediates homo-oligomerization by tetramer formation and serves as binding interface for ETO family members. Current studies propose the NHR2 domain as the only essential ETO domain crucial for AML1/ETO leukemogenic potential. Thus, selective interference with the oligomerization domain could inhibit the leukemogenic potential of AML1/ETO. Recently we could demonstrate that the lentiviral delivery of NHR2-derived peptides to AML1/ETO transformed cells abrogated AML1/ETO oligomerization leading to the transcriptional activation of repressed AML1 target genes. The intracellular expression of peptides in AML1/ETO dependent cell lines as well as in AML1/ETO immortalized human CD34 progenitor cells led to differentiation and cell cycle arrest arguing for a central role of the NHR2 domain for the maintenance of AML1/ETO transformation. Further we can show that an 18-mer short peptide derived from the NHR2 sequence, which is able to contact both alpha helical strands on the dimer surface, is able to disrupt tetramer formation at high molar ratios. In the presence of the 18-mer peptide, the percentage of tetrameric AML1/ETO molecules is clearly reduced leaving mostly monomers and dimers of AML1/ETO. Mutation of critical contact amino acids within the 18-mer peptide abolished these effects. The 18-mer peptides will be further optimized for binding properties and might be used as leading structures for drug development.

Identification and functional characterization of a novel mutation in the mortalin/GRP75 gene in German Parkinson disease patients

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We identified mortalin/GRP75 as a mitochondrial DJ-1-interacting protein that is responsible for cellular stress response and has been linked to Parkinson's disease pathogenesis due to significantly reduced levels in affected brain regions of PD patients indicating a potential role in neurodegeneration. To define the relevance of mortalin/GRP75 in DJ-1-mediated mitochondrial protection we performed a detailed mutation analysis in 286 German sporadic and familial PD patients and subsequent functional studies in PD.

Genes identified in PD encode proteins involved in the maintenance of mitochondrial homeostasis and oxidative stress response and lead, when mutated, to increased sensitivity for the onset of neurodegeneration. Loss of DJ-1 function leads to reduced mitochondrial membrane potential suggesting potential relevance of its interaction with mortalin/GRP75 for mitochondrial homeostasis.

Using DHPLC analyses for high throughput mutation screening and subsequent direct sequencing, we defined sequence variations that were compared to 290 healthy controls and analyzed them concerning differential interaction, mitochondrial morphology and mitochondrial function, ROS production and cell death in different cellular models.

One mutation in exon 12 in the mortalin/GRP75 gene, not present in more than 500 control chromosomes, lead to an amino acid exchange in a highly conserved region. Using immunoprecipitation we found no evidence for differential interaction of the mutant protein with DJ-1. However our functional studies argue in favour of subcellular mislocalization, a higher susceptibility towards proteolytic stress as well as decreased cell viability caused by mutant mortalin/GRP75 in stably transfected HEK293 cells.

This novel mutation in the human DJ-1-interacting mortalin/GRP75 gene links this mitochondrial protein with PD. Future studies focus on functional effects of the identified variant in neurons to determine the relevance of mortalin/GRP75 in neurodegeneration.

Role of single heterozygous PINK1 mutations as a susceptibility factor in Parkinson disease: Evidence from a family study including clinical and neuroimaging investigations

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Homozygous or compound heterozygous mutations in the PTEN-induced putative kinase 1 (PINK1, PARK6) are one cause of early-onset Parkinson disease (PD). The mode of inheritance is considered to be autosomal recessive. However, single heterozygous mutations in this gene have been suggested as a susceptibility factor for PD. In keeping with this notion, we previously reported a large German family with four homozygous mutation carriers (c.1366C>T) who were all definitely affected with PD. Furthermore, six of their 11 heterozygous offspring were identified with signs of probable (n=2) or possible (n=4) PD.
To further evaluate the role of heterozygous PINK1 mutations, we performed a clinical follow-up of all 10 available heterozygous mutation carriers from this family three years after the initial visit including detailed neurological examination, smell and color discrimination testing as well as neuroimaging by positron emission tomography (PET). The clinical and PET data were compared between homozygous and heterozygous mutation carriers as well as to healthy controls.
While there was mild worsening of clinical signs in previously affected heterozygous mutation carriers upon follow-up, three additional individuals had newly developed signs of possible PD. Hyposmia was found in seven of the heterozygous mutation carriers, diminished color discrimination in four. While the definitely affected homozygous mutation carriers showed a severe, 60% decrease of caudate and putaminal FDOPA uptake, heterozygous offspring also had a significant 20% putaminal FDOPA uptake reduction compared to controls.
These findings strengthen the hypothesis that heterozygous PINK1 mutations act as a susceptibility factor to develop at least subtle PD motor and non-motor signs, as supported by the finding of a reduced striatal dopaminergic FDOPA uptake not only in homozygous but also, albeit to a lesser extent, in heterozygous mutation carriers.

Genome-Wide Association Study reveals genetic risk underlying Parkinson's disease

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Background: Major advances in genotyping technology have allowed rapid genome-wide screening of common variants in large populations and launched a new era in the investigation of the genetic basis of complex diseases. Parkinson's disease (PD) has a largely unknown etiology, but epidemiological studies highlight a significant genetic contribution to disease risk.
Objective: To perform a genome-wide association study (GWAS) in PD.
Methods: In a first stage 561,467 SNPs were genotyped in 1,713 Caucasian patients with PD and 3,978 controls. The replication stage included 3,513 cases and 4,710 controls.
Results: Two strong association signals were observed: in the a-synuclein gene (SNCA) (rs2736990, OR=1.23, p=2.69x10-16) and at the MAPT locus (rs393152, OR=0.77, p=5.14x10-17). We exchanged data with colleagues performing a GWAS in Asian PD cases. Association at SNCA was replicated in the Asian GWAS, confirming this as a major risk locus across populations. The association at MAPT was absent in this cohort, indicating population specific genetic heterogeneity in this complex disease. We were able to replicate the effect of a novel locus detected in the Asian cohort (PARK16, rs823128, OR=0.66, p=1.32x10-7) and provide evidence supporting the role of common variability around LRRK2 in modulating risk for PD (rs1491923, OR=1.14, p=2.10x10-5).
Conclusions: These data demonstrate an unequivocal role for common genetic variability in the etiology of typical PD.

Olfactory function in mouse models of Parkinson´s Disease

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Non-motoric symptoms in Parkinson´s disease (PD) such as olfactory dysfunction and cognitive impairments are often occurring before the cardinal motoric symptoms and affect large portions of PD patients. However, in genetic mouse models of the disease these symptoms are rarely assessed. Therefore we set up a behavioural test-battery to specifically assess for these symptoms in genetic mouse models of PD generated in our institute . First, we assess social recognition memory in a discriminative test version before subjecting them to a more detailed exploration of olfactory function using a modified version of a positively reinforced olfactory conditioning procedure. Thereafter we test for cognitive function using an object recognition test. We tested three genetic animal models of PD generated at out institute, Pink1 knock-out, Lrrk2 knock down and DJ-1 deficient mice.
Pink1 null male showed a clear social discrimination deficit in the absence of any detectable alteration in motor function. This phenotype was accompanied by a deficit in discriminatory abilities and reduced sensitivity in our olfactory test battery, while object recognition memory was intact in Pink1-/- mice. In contrast Lrrk2 knockdown animals only displayed reduced olfactory sensitivity, whereas DJ-1 deficient animals were not impaired in olfactory function but male mutants showed a clear cognitive phenotype and slight impairments in motoric function. These results indicate that Pink1, Lrrk2 and DJ-1 probably exhibit functions outside the dopaminergic system and beyond dopaminergic neurotransmission, a notion conceivable due to the widespread expression of these genes in the brain.

The picornavirus peptide T2A as a mechanism for ADAM10-promoter reporter mouse generation

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The amyloid precursor protein (APP) which is closely connected to the pathogenesis of Alzheimer's disease (AD) is processed by different secretases: one is the a-secretase ADAM10 which cleaves within the amyloid-ß sequence of APP, thus preventing the release of toxic Aß-peptides and generating neuroprotective and neurotrophic APPsa. Therefore, ADAM10 presents a valuable target in therapeutic treatment of AD. One strategy is to enhance the amount of cellular ADAM10 due to the activation of its gene expression. In cell culture studies and in AD model mice, retinoic acid has been demonstrated as a potent inducer of ADAM10 gene expression. For further elucidating aspects of ADAM10 gene regulation in vivo, we intend to generate a reporter mouse with a luciferase as an indicator for endogenous murine promoter activity.
For initial experiments in cell culture, a new construct was cloned including the human ADAM10-promoter, the cDNA for the firefly-luciferase, a picornavirus-T2A-peptide coding sequence and the cDNA of bovine ADAM10. Picornaviridae have a special mechanism for synthesis of single proteins stemming from one joint mRNA during translation: in between the sequences, coding for the capsid and the replicative proteins of the virus, a sequence is located which encodes the 2A-peptide. This peptide induces a 'ribosome-skipping'-effect during translation which disrupts peptide bond generation and leads to biosynthesis of two independent proteins from one mRNA. Tests were performed in either human or murine neuroblastoma cells with the new plasmid, to examine the 'ribosome-skipping'-effect and activity of both enzymes, ADAM10 and the luciferase. Our results show, that the T2A-peptide does not impair firefly-luciferase activity and leads to a functional ADAM10 in the neuronal cell lines. Both enzymes are synthesized from a joint mRNA and the approach using the 2A-peptide for generating an ADAM10 promoter-reporter mouse, thus seems to be promising.

Promoter analyses of Alzheimer´s disease-related genes ADAM10, BACE1 and APP

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The proteolytic cleavage of the amyloid precursor protein (APP) by the beta-secretase BACE1 (beta-site APP cleaving enzyme 1) leads to the generation of neurotoxic amyloidogenic peptide Aß. The Aß-peptide is the main component of amyloid plaques, which can be found in the brain of Alzheimer´s disease (AD) patients and initiates neuronal cell death. On the contrary, the proteolytic processing of APP by the alpha-secretase ADAM10 precludes the formation of Aß-peptides and amyloid plaques via cleavage within the Aß sequence.
Thus, modulating the ratio of alpha-secretase to beta-secretase activity is a promising target for a therapeutical approach against Alzheimer´s disease. In this context it is imperative to increase the enzymatic potential or the amount of ADAM10 and decrease the BACE1-activity. One possible strategy for the modulation is the up-, respectively down-regulation of gene expression.
Our first aim is the identification of substances with the property of specifically up-regulating ADAM10 gene expression. For this purpose, we are establishing a cell-based high-throughput promoter assay to screen various drugs and candidate proteins. Therefore we cloned the human promoters of the AD relevant key-genes ADAM10, BACE1 and APP and integrated them into a luciferase reporter vector. Initially we examined the basal activity of either promoter in four different human neuronal cell lines. In the following, we analyse the regulatory effect of more than 500 different transcription factors on the human ADAM10- and BACE1-promoter activity. For further in vivo evaluation of ADAM10 gene expression activators identified in the promoter assay, a mouse reporter model is being generated.

EGCG is a potent remodeling agent of mature amyloid fibrils and reduces cellular toxicity

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Recently, we demonstrated that the polyphenol (-)-epigallocatechingallate (EGCG) inhibits alpha-synuclein (AS) and amyloid-beta (Abeta) fibrillogenesis. It associates with natively unfolded polypeptides and promotes the self-assembly of unstructured oligomers of a new type. Whether EGCG disassembles preformed amyloid fibrils, however, remained unclear. Here, we show that EGCG has the ability to convert large, mature AS and Abeta fibrils into smaller, amorphous protein aggregates that are non-toxic for mammalian cells. Mechanistic studies revealed that the compound directly binds to beta-sheet rich aggregates and mediates the conformational change without their disassembly into monomers or small diffusible oligomers. These findings suggest that EGCG is a potent remodeling agent of mature amyloid fibrils.

Genetic Variants of DTNBP1 (Dystrobrevin Binding Protein 1 / Dysbindin) – a Schizophrenia Susceptibility Gene – are associated with Altered Hippocampal Glutamate Concentrations in Healthy Individuals

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Introduction: In several association and linkage studies the DTNBP1 gene has been identified as a major susceptibility gene for schizophrenia. Reduced expression of DTNBP1 was found in the hippocampus and prefrontal cortex in post mortem brains of schizophrenic patients. In vitro and animal models provide evidence that DTNBP1 gene product Dysbindin modulates the activity of the neurotransmitter glutamate in hippocampal neurons. This study is the first to investigate the effects of genetic variants of DTNBP1 on glutamatergic concentrations in the hippocampus and a cortical region, the anterior cingulate cortex (ACC) in humans.
Methods: In 79 healthy subjects, the association of single nucleotide polymorphisms rs760665, rs909706 and rs6926401 with absolute concentrations of glutamate in the left hippocampus and ACC were explored, using proton magnetic resonance spectroscopy at 3 Tesla.
Results: Hippocampal glutamate concentrations were significantly affected by genotype of rs760665 [A/T] and rs909706 [C/T]. Regarding both polymorphisms, TT-carriers showed lower glutamate concentrations than the carriers of the other genotypes. Glutamate concentrations in the anterior cingulate cortex were unaffected by genotype. Statistical analysis of rs6926401 was not suitable due to the unbalanced genotype distribution.
Conclusion: The results support a role of DTNBP1 gene variants on hippocampal glutamate concentrations in healthy humans and are in line with the findings of in vitro and animal models.

fMRI cue-reactivity as an endophenotype of alcohol-dependence: Paradigm development and validation

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In alcohol-dependent individuals, relapse to compulsive alcohol consumption occurs even after years of abstinence; often in response to encountering alcohol-related stimuli or contexts. Functional neuroimaging studies suggest that, even when abstinent, dependent patients react with increased brain activations in areas of the mesocorticolimbic "reward" circuit to alcohol-associated cues. Individual variability in the magnitude of fMRI cue-reactivity (especially within reward-related areas) seems to be, at least in part, genetically modulated. In order to utilise fMRI cue-reactivity as an endophenotype of alcohol-dependence, the development of a valid and reliable neuroimaging paradigm is of utmost importance.

A cue-reactivity experiment using a novel set of craving-eliciting alcohol pictures was developed and tested in 31 recently detoxified alcohol-dependent patients. Using a 3 Tesla MRI scanner, functional imaging data were collected twice, once at 5 to 21 days after detoxification and once 3 weeks after the first measurement. The results indicated that alcohol relative to neutral visual stimuli elicited increased activations in mesocorticolimbic areas previously implicated in cue reactivity studies [e.g. putamen/ striatum (motivation/ reward processing); occipital and parietal regions (attentional/ visual processing); posterior cingulate cortex (emotion processing)]. It was also found that fMRI cue-reactivity decreased with increasing duration of abstinence, but that activations in the same brain regions could still be observed three weeks after the first measurement point. These results suggest that the paradigm is a suitable tool for a genotype-endophenotype association approach such as the commencing NGFNplus "Genetics of Alcohol Addiction" project "Endophenotyping with fMRI: Genetic modulation and treatment response".

Improved Long-Term Memory Performance for Reinforced Reward-Prediciting Stimuli - A Pilot Study.

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Converging evidence suggests that the hippocampus-dependent episodic memory encoding is modulated by dopaminergic midbrain signals, which in turn are under prefrontal glutamatergic control. Reward-predicting stimuli are thus preferentially encoded into long-term memory. A related mechanism might be involved in alcoholism, with alcohol-dependent patients attributing atypically high salience to addiction-related stimuli.
12 healthy subjects participated in a reward prediction task. 60 outdoor and 60 indoor scenes (predicting reward vs. neutral outcome) were presented, 50% showing alcoholic and 50% non-alcoholic beverages. Fast and correct responses (66%) in a following digit-classification task, were rewarded with 0.5€ in the reward condition. After 24 hours, participants were shown the original cues, intermixed with novel pictures, and asked to perform a 4-level forced choice recognition task. Data analysis was conducted using SPM8 and standard two-stage GLM statistics.
No significant behavioural effect of condition on recognition was found. However, within the reward condition, pictures followed by successful gain were significantly better recognized than pictures followed by reward omission ($p = .002$). The ventral anterior cingulate (vACC) and the left nucleus accumbens (NAcc) showed significantly higher BOLD responses for reward-predicting relative to neutral stimuli ($p<0.005$). In the left hippocampus, a correlation between memory performance and BOLD response was found ($r2=0.67$, $p < .05$, corrected). However, no condition x memory interaction was detected.
Notably, the behavioral results were only in part mirrored by the neural activations. Compatible with the model of Lisman and Grace, a reward effect in the vACC and NAcc and a hippocampal memory effect were found, but no interaction was observed. However, given the small sample size and high interindivdual variability of memory performance, the study might currently be underpowered to detect such an interaction.

Involvement of the atrial natriuretic peptide transcription factor GATA4 in alcohol dependence, relapse risk, and treatment response to acamprosate

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Identifying genes contributing to relapse and treatment response will improve our understanding of the mechanisms underlying alcohol dependence and will yield treatment targets for personalized medicine. We sought to determine whether 15 single nucleotide polymorphisms (SNPs), linked to alcohol dependence by a recent genome-wide association study (GWAS), are associated with relapse behavior. Moreover we examined whether those associations can be linked to pharmacological treatment response. In 374 participants with a DSM-IV diagnosis of alcohol dependence that underwent a randomized, double-blind, placebo-controlled trial with acamprosate, naltrexone or placebo, fifteen SNPs were genotyped and tested for an association with relapse during twelve weeks of treatment. Treatment response with naltrexone and acamprosate was tested for association with these genetic variations. SNP rs13273672, an intronic SNP in GATA4, was significantly associated with relapse within a 90-days medical treatment period ($P < .01$). Subsequent pharmacogenetic analysis showed that this association was mainly based on patients treated with acamprosate ($P < .01$). In line with the observation that natriuretic peptide promoters are modulated by GATA4 a significant gene dose effect in ANP plasma concentration in the different GATA4 genotypes($P = .05$) is demonstrated. Thus genetic variations in GATA4 might influence relapse and treatment response to acamprosate in alcohol dependent patients via modulating ANP plasma levels. If replicated, these results would help to identify alcohol dependent patients who may be at high risk of relapse and better respond to treatment with acamprosate.

Results from genome wide association screens for alcohol consumption in the general population

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In longitudinal epidemiological studies moderate alcohol consumption is mostly associated with favorable outcomes wheras abstinence as well as marked consumption is associated with various risks. Alcohol consumption is partly determined by genetic factors. Data were analysed from two large genome wide screens comprising 1623 (Affymetrix 500 k) and 1776 population based individuals (Affymetrix 1000K). For the combined analysis, missing SNPs were imputed. Alcohol consumption (g/kg/day) was used as a quantitative variable. Some SNPs wee replicated in up to 3287 individuals.
The best combined p-value of any SNP was 7.29E-08. Eight SNPs hat a combined p-value between 1.78E-07 and 8.24E-07. Thus, at least one SNP reached nominally global significance.

Molecular genomics of intracellular calcium handling in diastolic dysfunction, heart failure and arrhythmias

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Intracellular calcium dysfunction in the heart is an accepted mechanism of maladaptive remodeling contributing to contractile dysfunction and arrhythmias. In heart disease several calcium handling genes undergo significant changes in expression and/or function. We aim to identify changes in and mechanisms of dysregulated gene expression associated with intracellular calcium leak from defective cardiac ryanodine receptors (RyR2s). RyR2 calcium leak is a molecularly defined mechanism of cardiac remodeling, however, the pathways are not well understood. We characterize the role of catecholamines (Isoproterenol) and peptide hormones (Angiotensin II) on cardiac remodeling and changes in gene expression in wild-type and RyR2-mutant mice with calcium leak. As an exploratory study, wild-type mice received isoproterenol continuously by osmotic minipumps for 3 or 10 days (20 mg/kg/d s.c.). After three (Isoproterenol) and ten days (Isoproterenol and Angiotensin II) hearts were harvested, Langendorff perfused and total RNA was extracted. Gene expression was analyzed by gene arrays. Whole transcript expression (GeneChip® Mouse Gene 1.0 ST Array; Affymetrix) was compared between placebo and agonist treated animals. We identified genes that showed significant changes in expression in agonist treated animals compared to the placebo group, which was confirmed by quantitative RT-PCR analyses and will be presented. The results confirm our study design which is going to be extended to RyR2 knockin mice with missense mutations linked to stress-induced syncope and arrhythmias. In a parallel approach we plan to investigate changes in microRNA levels using microarrays, quantitative RT-PCR and Northern hybridization analysis in order to identify pathways of mRNA regulation associated with RyR2 calcium leak. Depending on the results, selected microRNAs will be further characterized by viral overexpression assays and interference with anti-sense RNA constructs for target identification.

New set of potential tumor marker by miRNA Profiling in human Pancreatic Cancer

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Pancreatic cancer is the fifth most frequent cause of cancer-related death in industrialized countries. The diagnosis of ductal adenocarcinoma of the pancreas is associated with poor prognosis, an increasing incidence and no or only ineffective means of treatment. MicroRNAs are a recently discovered class of small non-coding RNAs that have been reported to play an important role in cell growth and apoptosis, differentiation and tumor progression. microRNA profiling seems to have the potential being a promising clinical diagnostic, prognostic and therapeutic tool for cancer

In this study, we are establishing microRNA analyses using the Geniom® Biochip miRNA homo sapiens with 847 human microRNAs. We analysed samples of pancreatic ductal adenocarcinoma and compared them to the results obtained with normal pancreatic cells and pancreatitis.
In summary, we have discovered a number of miRNAs that are differentially expressed in pancreatic cancer. Classification of tumor and non-tumor patients of the pancreas was realized by different statistical tools.

The resulting diagnostic DNA-chip will be of significant clinical utility to detect cancer cells in tissues and whole blood from patients with pancreatic carcinoma and to draw prognostic conclusions based on their molecular appearance. Combining this data with clinical information permits the definition of sub-groups within an analysed cohort and may provide a robust means for diagnosis and prognosis.

Random Jungle: A fast implementation of Random Forest for high dimensional data

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Genome-wide association studies (GWAs) have proven to be a successful approach for unravelling the genetic basis of complex genetic diseases. However, the identified associations are not well suited for disease prediction, and only a modest portion of the heritability can be explained. This may partly be due to the weak ability of standard statistical approaches to detect gene-gene and gene-environment interactions. A promising alternative are random forests which have already been successfully applied in candidate gene analyses. Important single nucleotide polymorphisms are detected by permutation importance measures, and we show that these differ depending on the used implementation. To this day, the application to GWA data was impossible with existing implementations because of the high computational burden. Here, we present the new freely available software package Random Jungle which facilitates the rapid analysis of GWA data. The new tool yields valid results and computes up to 182 times faster than the fastest alternative implementation, while still maintaining all options of other programs. Specifically, it offers the different permutation importance measures available. It includes new options like a backward elimination method. The application of Random Jungle to GWA data is presented.

Genetic variations in the NLRC4 gene influence IL-18 levels

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Background Interleukin-18 (IL-18) is a cytokine with complex immune-modulatory functions. A potential role of the IL-18 system in cardiovascular disease has been suggested. Several studies have linked genetic variation in the IL-18 system to circulating biomarker concentrations and disease susceptibility, but have remained controversial.
Caspase-1 is required for processing of IL-18 and activation of caspase-1 is mediated by the inflammasome via CARD-CARD domain interaction. NLRC4 (NOD-like receptor), a CARD-containing protein, is a critical component of the inflammasome and caspase-1 activation. The aim of this study was to further explore the genetic basis of IL-18 and its relation to cardiovascular diseases.
Methods A meta-analyses of GWAS was performed in the Gutenberg Heart (n = 2,930) and Framingham Heart Study (n = 2,940). Replication was performed in PRIME (n = 1,140) and FinRisk (n = 8,050). Clinical implication of results was evaluated in the AtheroGene Study (n = 1,761).
Results A strong association was found for a locus on chromosome 2 within NLRC4 (top SNP p=3.0x10-28) and decreased IL-18 levels. The genetic variation was also associated with a decreased incident cardiovascular death in patients with coronary artery disease (HR (95%CI) 0.71 (0.54-0.94), p = 0.015).
To investigate the functional consequences of the genetic variations within NLRC4, caspase-1 activity and IL-18 levels were determined in healthy individuals (n = 398). These determinations showed that caspase-1 activity is reduced in individuals containing the allele associated with reduced levels of IL-18.
Conclusions By GWAS we identified a locus on chromosome 2 related to levels of IL-18. This locus within the NLRC4 gene was also associated with cardiovascular death in patients with coronary artery disease. The underlying mechanism of the genetic variants on IL-18 levels involves reduced caspase-1 activity, suggesting an effect on CARD-binding of NLRC4 and thereby activation of caspase-1.

TNFa-M-Ras-LFA-1 pathway analysis in genome-wide SNP data on patients with myocardial infarction

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BACKGROUND: Genome-wide association studies (GWAS) typically focus on single-locus analysis. By contrast, systematic analysis of an affected pathway may be more informative. Recently, we identified the MRAS gene as a new risk gene for CAD/MI (rs9818870 P=7.44 x 10-13 (OR=1.15; 95% CI=1.11–1.19) by GWAS. The underlying biology is unclear.
AIM: We aimed to perform a MRAS gene based pathway analysis (TNFa-M-Ras-LFA-1 pathway) using Affymetrix genotype data from the German MI Family Studies (GerMIFS I, GerMIFS II, and GERMIFS III (KORA)) comprising a total of 2,000 MI cases and 3,000 population-based controls.
METHODS: Identification of relevant genes was undertaken by bioinformatical means of medical literature [pubmed] and internet databases [Ensembl, SNPper]. Available SNPs were investigated in silico in three independent genome-wide SNP data sets genotyped on Affymetrix platforms; missing genotypes were subsequently imputed (GerMIFS I [875 MI cases, 1644 controls] and GerMIFS II [1221 MI cases, 1298 controls].
RESULTS: Thirteen genes were identified in TNFa-M-Ras-LFA-1 pathway. Among them, 7 genes carried several SNPs significantly associated with CAD/MI in both GWAS. In ICAM-1 17/70 SNPs, in ITGAL 8/47 SNPs, in ITGB2 25/147 SNPs, in MRAS 43/ 91 SNPs , in Rap1A 29/276 SNPs, in RAPL 30/194 SNPs, in RIAM 16/228 SNPs, TNFa 13/140 SNPs, in TNFRSF1A 7/56 SNPs and in TNFRSF1B 14/91 SNPs were significant for CAD/MI [combined p-values ranging from 0.001 – 5x10-5).
CONCLUSIONS: Several SNPs in genes of the TNFa-M-Ras-LFA-1 pathway revealed significant results for CAD/MI in two GWAS. Replication steps are needed to further establish the role of this pathway in the etiology of CAD/MI.

Associations between variations in the TLR4 gene and incident coronary heart disease (CHD) in middle-aged men and women: Results from the MONICA/KORA Augsburg case-cohort study, 1984-2002

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Purpose - Toll-like receptor 4 (TLR4), the signalling receptor for lipopolysaccharides, is an important member of the innate immunity system. Since several studies have suggested that atherosclerosis might be associated with changes in the innate immune response, and a SNP in the TLR4 gene (Asp299Gly; Kiechl et al, N Engl J Med 2002;347:185-92) was shown to be associated with a decreased risk of atherosclerosis, we sought to investigate the impact of gene variants in the TLR4 gene on incident CHD.
Methods - A case-cohort study was conducted in initially healthy, middle-aged men and women based on data from the MONICA/KORA Augsburg studies collected between 1984 and 2002, with a mean FU of 10.2±4.8 years. The present analyses are based on 318 case subjects with incident CHD and 1,727 non-case subjects. Seven SNPs (rs2770150; rs6478317; rs1927911; rs2149356; rs4986791; rs7873784; rs1927906) were systematically selected in the TLR4 gene, and haplotypes were constructed.
Results – TLR4 genotype distribution did not significantly differ among subjects with incident CHD and non-case subjects. No significant interactions between any of the SNPs and major cardiovascular risk factors on CHD risk were found. There was no consistent association between the 7 different SNPs within the TLR4 gene and incident CHD in crude and in multivariable adjusted analyses, neither for men and women separately, nor in a model that included all study participants.
Conclusions – In contrast to an earlier and smaller prospective study, we could not confirm an association between various SNPs within the TLR4 gene and incident CHD. The presence of various alleles of the TLR4 gene, including Asp299Gly, does not seem to exert a major influence on the progression of atherosclerosis in the general population.

Genome-wide association study on HDL subclass traits

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HDL cholesterol is an established prognostic marker of cardiovascular events. However, HDL is not a homogenous class, but can be further divided into subclasses. Heritable large HDL particles have been associated with a favorable cardiovascular risk profile. The regulation of HDL subclass distribution is not fully understood. We therefore conducted a genome-wide association study with traits of the HDL subclass profile in subjects of the German MI family study. Methods: Genotypes and NMR spectroscopy measurements of lipid profile were available for 843 individuals. We analyzed the traits HDL mean particle size and HDL particle number in relation to genotypes derived from genome-wide arrays (Affymetrix 500k, Affymetrix 5.0 and Affymetrix 6.0). Association analyses were performed assuming an additive model of inheritance and adjusted for age and gender. Results: With respect to HDL particle size and particle number, the lowest p-values had a magnitude of 2 x 10-5. Associations at p < 10-4 were found for 28 SNPs (HDL particle size) and 9 SNPs (HDL particle number), respectively. Conclusion: In the German MI family study none of association tests with HDL particle number and particle size individually attained experiment-wide significance, suggesting that our dataset may be underpowered for genome-wide analyses. More definitive analyses are warranted.

Coronary ARtery DIsease Genome-wide Replication And Meta-Analysis (CARDIoGRAM) - Design of a prospective meta-analysis of 14 genome-wide association studies

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CAD is the leading cause of death in U.S. and Europe. GWAS have uncovered at least 13 common alleles associated with CAD. However, each variant confers a modest effect, and together, the variants explain a small fraction of heritability. These observations suggest that additional loci harboring CAD-associated variants might be discoverable with larger samples and improved statistical power. To accomplish this, we assembled CARDIoGRAM that pools GWAS data from ADVANCE, CADomics, CHARGE, deCode, GerMIFS I-III (KORA), LURIC/AtheroRemo, MedStar/PennCath, MIGen, OHGS, and the WTCCC. In total, the consortium comprises more than 22,000 well-characterized cases with CAD and more than 60,000 controls. In each individual study, genome-wide genotyping was carried out on either Affymetrix or Illumina platforms, and imputation was conducted to generate genotypes for 2.2 million SNPs in each study.

With the aim of conducting a type II meta-analysis using GWA results from the single studies and consortia, SOPs were generated in order to harmonize the QC and data analyses. Extensive QC was performed both study-wise and centrally so as to provide standardized data formats of high quality. With the assembled sample size, the estimated power to detect modest effects is substantially increased. For instance, even for genome-wide significance, the power is about 80% for an odds ratio of 1.1, provided that the minor allele frequency is at least 10%.

Meta-analyses for CAD phenotype as well as for important subgroups including myocardial infarction and early-onset CAD will be carried out. Following the initial evaluations, wet lab replication genotyping of top results will be sought in more than 15,000 additional cases and 15,000 controls. CARDIoGRAM brings together an enormous wealth of GWA studies data on CAD and myocardial infarction, thus representing the largest study to date to uncover the inherited basis for the leading public health problem in the industrialized world.

1.3Mb deletion within chromosome band 21q22.3 associated with features of Williams-Beuren syndrome

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We report on a patient with a de novo microdeletion within chromosome band 21q22.3 detected by array CGH analysis. The 12 years old girl demonstrated with some features resembling Williams-Beuren syndrome with microcephaly, slightly upslanting palpebral fissures, broad nasal tip, tooth anomalies, hoarse voice, and mild motor but more significant mental delay. The Williams-Beuren syndrome (WBS; MIM 194050) is caused by a hemizygous contiguous gene deletion on chromosome 7q11.23 and is characterized by typical facial dysmorphisms, congenital heart defects, hypercalcemia, hoarse voise, dental malformations, growth retardation, and mental retardation inter alia.
The patient showed an apparently normal female karyotype on conventional cytogenetic analysis. A microdeletion of the WBS critical region 7q11.23 could not be detected by fluorescence in situ hybridisation (FISH). Furthermore, subtelomeric rearrangements were excluded using the ToTelVysion set (Abbott). However, array CGH analysis uncovered an interstitial 1.3Mb deletion within chromosomal region 21q22.3. This deletion encompasses 17 genes. FISH analysis confirmed this result and the deletion was excluded in both parents.
Three patients with a larger 21q22.3 deletion and with some symptoms described, similar to our patient, are published in the Decipher database (https://decipher.sanger.ac.uk). Thus, it seems possible that 21q22.3 microdeletions may cause a recognizable phenotype including mental retardation. Further analyses of cases with 21q22.3 deletions are necessary to characterise the spectrum of clinical findings in these patients.

Definition of the critical region in chromosome band 1q25 for patients with mental retardation and cleft lip or palate.

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Microdeletions and microduplications are an established mechanism for causing mental retardation with and without further clinical findings. Interstitial deletions on the long arm of chromosome 1 have so far been classified as either proximal deletion ranging from 1q21 to 1q25or intermediate deletion from 1q24 to 1q32. The phenotypes of the patients in each group overlap partially (Descartes et al., 2008).
Here, we report on one patient with an interstitial deletion in 1q25.2-q25.3 combined with duplication in 1q25.2. The four-year-old girl was referred to the clinical genetics center because of mild/moderate developmental delay, cleft palate, microcephaly, epilepsy, and mild facial dysmorphic features. The unspecific combination of the features did not allow for a diagnosis. Therefore, whole genome array CGH using an Agilent 244 A chip was performed, with the result of a de novo duplication accompanied by a de novo deletion in chromosome band 1q25 (arr 1q25.2 (174.437.033-175.260.375x3), arr 1q25.2-q25.3 (175.273.320-182.120.888x1).
All features present in our patient have been reported in other patients with larger interstitial deletions including band 1q25 and demonstrate the minimal clinical presentation of this syndrome. It was therefore possible, based on our data, to reduce the smallest region of overlap (SRO) from 14 MB to 6.5 Mb. Moreover, 1q25.2-q25.3 may be considered as a new cleft palate associated locus, since over 70% of the patients with overlapping interstitial deletions in 1q25 have lip and or palate anomalies. The definition of the candidate gene is still difficult, since the overlapping region has a size of 6.5 Mb and contains 48 genes with many of them of unknown function.
We expect that the MRNET project allows for identifying further patients with 1q25 deletions. This will either provide evidence to classify the 1q25 deletion as a contiguous gene syndrome or to define single genes causing lip/palate abnormalities and developmental delay.

Mutations in MEF2C from the 5q14.3q15 microdeletion syndrome region are a relatively frequent cause of severe mental retardation and diminish MECP2 expression

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The etiology of mental retardation remains elusive in the majority of cases. Microdeletions within chromosomal bands 5q14.3q15 were recently identified as recurrent cause of severe mental retardation, epilepsy, muscular hypotonia, variable brain and minor other anomalies. By molecular karyotyping we identified two novel 2.4 and 1.5 Mb microdeletions of this region in patients with a similar phenotype. Both deletions contained the MEF2C gene, which is located proximally to the previously defined smallest region of overlap. Nevertheless, due to its known role in neurogenesis, we considered MEF2C as a phenocritical candidate gene for the phenotype associated with 5q14.3q15 microdeletions. We therefore performed mutational analysis in 362 patients with severe mental retardation of unknown etiology. Within this cohort we found 2 truncating and 2 missense de novo mutations in MEF2C which establishes this transcription factor as a novel relatively frequent autosomal dominant cause of severe mental retardation accounting for as much as 1.1% of patients. By using a transcriptional reporter assay we show that MEF2C mutations diminish synergistic transactivation of E-box promotors including that of MECP2, for which we found diminished expression by RT-PCR in vivo. We therefore hypothesize that the phenotypic overlap of patients with MEF2C mutations and atypical Rett syndrome is due to the involvement of a common pathway.

MRNET TP7 – Summary of high resolution molecular karyotyping of patients with mental retardation – Identification of regions containing candidate genes

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The German Mental Retardation Network (MRNET) is a nationwide consortium that aims for elucidation of genetic causes of intellectual disability (ID). Currently, ten centers (TP="Teilprojekt") are involved. Participating patients are carefully examined in the clinical genetics departments of each center. Eligible causes like Fragile X syndrome or RETT disease as well as other phenotypically noticeable syndromes are excluded by standard diagnostics before chromosome analysis is performed. If this examination does not reveal any major genomic rearrangements array CGH is carried out. We are using Agilent's array CGH platform which is at present being updated to the 1M chip and has been used with the standard 244k chip so far. Every suspicious copy number variation (CNV) is validated by FISH analysis, customized array CGH, quantitative PCR, or quantitative genotyping in patients as well as in parents to see if a certain variation is of familial origin or arose de novo. All obtained data sets are fed into a database that is accessible for every participating center to carry out comparisons to distinguish between unpublished common CNVs and CNVs that contain candidate genes. Disease causing mutations that have been confirmed as plausible reason of a patient's feature will be further investigated for functional analysis for example in animal models. Out of 78 patients we found eight de novo mutations and 17 patients without suspicious CNVs while all others carry familial rearrangements of unclear meaning. Candidate loci we are currently investigating include one locus with four genes for frontotemporoparietal polymycrogyria and one gene for perisylvian polymicrogyria, one candidate gene for Shprintzen-Goldberg syndrome/Loeys Dietz syndrome type 1, one gene for microcephaly as well as several other genes for non-syndromal intellectual disability.

A data management tool for Agilent array CGH export files based on a GRAILS application framework

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Performing array CGH has become a standard analysis in many facilities that deal with genetic diseases or with genomic rearrangements in humans or in animal models. A major task of this routine is data handling. The basic output file of an Agilent array CGH analysis is a CSV table that contains the chromosomal/genomic position of the first and last affected probes of a copy number variation (CNV), how many probes are affected, and the p-values of amplifications or deletions. Not every CNV detected is considered relevant for the outcome of the analysis. Therefore, it seemed very useful to have an application that stores annotations about validation procedures, already performed assessments, and importance of a certain CNV, provides options to perform database queries by a single click, offers different levels for grouping data sets, and is able to browse and search accumulated data on different levels to name some features. The program is a traditional web/database application, based on the open-source framework Grails (http://grails.org/). Aspects of the research data are modeled as tables in the database, and the framework provides standard views on the data (create/read/update/delete). Several of the views have been further customized to support additional features (custom reporting, linkaways to external databases, markup and annotation of the data). Using this tool for our daily work, we have been able to save money for personnel and to tremendously improve our data handling. We are willing to share this tool with other research labs.

Five new families with 15q13 microdeletion: Further delineation of the phenotypical spectrum and review of the literature

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Microdeletions of chromosome 15q13.2q13.3 are associated with a recurrent syndrome of mental retardation/ learning difficulties, seizures and behavioural problems.
Screening a cohort of 402 patients with mental retardation using a 250K or a 6.0 Affymetrix SNP array platform and analyzing further 184 patients by qPCR we identified 5 patients harbouring the typical 1.5 Mb deletion. In addition, we detected the aberration in 7 family members (4 parents and 3 siblings) by qPCR. Duplications in this highly unstable genomic region were not found in our cohort.
The deletion was inherited in all 4 index patients in whom parents were available. The index patients presented with mild to moderate mental retardation, especially language deficits, behavioural abnormalities and mild dysmorphic features but no recognizable phenotype. Additional features were conspicuous birth parameters and alterations in adult body height, weight, and head circumference as well as scoliosis, EEG changes or seizures and cardiac defects. Nevertheless, the microdeletion was associated with a highly variable intrafamilial phenotype. The frequency of this microdeletion in our cohort of mental retardation cases was 0.85% (5/586) and therefore slightly higher than the reported 0.3% ($p<0.06$). This suggests that the frequency of this 15q13.3 microdeletion disorder is comparable to that of the well known 1p36 microdeletion.

Detection of a 7Mb de novo deletion in a patient with distal Athrogryposis Type 2B (DA2B)

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We report on a 5 year old male patient presenting distal Athrogryposis with club feet, ulnar deviation and slight camptodactyly as well as facial dysmorphism including high forehead, small mouth, broad nasal bridge, epicanthus, high palate, brachycephalus, relative short neck, and dysplastic ears. Although motor development was delayed, speech and mental development was considered normal. The deformation of hands and feet and the facial features correlated with Type 2B of distal Athrogryposis according to the Bamshad classification (Krakowiak et al., 1998). Using an Affymetrix 250K Nsp Gene Chip array we found a 7Mb deletion located on chromosome 8q21.11- 8q21.13. In this region 662 SNPs and 24 refseq genes are situated. FISH analyses showed a de novo deletion of the BAC clone RP11- 89J14. Up to now mutations in three genes (MYH3, TNNT3 and TNNI2) are described as causal for the phenotype of distal Athrogryposis Type 2B. These genes encode for proteins that are relevant in muscle formation and contraction. In silico analysis of the gene content of the deletion showed that none of the 24 genes is directly involved in these processes. However, some of them are possible candidate genes because of their known function. For example ZFHX4, which encodes a homeodomain- zink finger protein, is published in connection with hereditary congenital ptosis (Nakashima et al., 2008), and possible involved in neural and muscle differentiation (Hemmi et al., 2006). This case shows that large genomic deletions are not necessarily leading to mental retardation but may cause any type of genetic disorder.

MOMO syndrome: an obesity mental retardation syndrome and further search for the disease causing mutation

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Two unrelated patients with a combination of macrosomia, macrocephaly, obesity and ocular abnormalities (retinal coloboma and nystagmus) were described by Moretti-Ferreira et al. (1993). Thereafter, the new syndrome - called MOMO syndrome (Macrosomia, Obesity, Macrocephaly, and Ocular abnormalities) - was categorized as an overgrowth syndrome. However, since a third patient, published in 2000, was of short stature, overgrowth was discussed as non-mandatory for the diagnosis.
We report here on a further patient with the proposed diagnosis of MOMO syndrome. At the age of 5 years and 7 months the girl presented with overweight, macrocephaly and borderline short stature. She demonstrated a prominent forehead, deep-set eyes, a flat nasal bridge, anteverted nares, a cupid bow upper lip, low-set posteriorly rotated ears and tapering fingers. She had hypotonia, recurrent febrile convulsions and developmental delay. A hand radiogram showed delayed bone age. Ophthalmological examination revealed a choroid coloboma in her left eye. Metabolic tests gave normal results.
Whole genome array CGH using an Agilent 244A chip detected a paternal deletion in chromosomal subband 15q22.3 in our patient. Further investigation will be carried out using next generation sequencing to clarify the cause of this rare syndrome.

A de novo 1q42 microdeletion of 1.17 Mb in a patient with a phenotype out of the neuro-cardio-facio-cutaneous spectrum disorders

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We identified a 3-year-old boy with clinical features similar to patients with neuro-cardio-facio-cutaneous (NCFC) spectrum disorders, including Noonan, Costello and cardio-facio-cutaneous syndrome. These autosomal dominant conditions are characterized by facial dysmorphism, congenital cardiac defects, reduced postnatal growth, and variable cognitive impairment as major features. The biological consequence of gene mutations associated with this group of diseases is predominantly increased signal trafficing through the RAS-RAF-MAPK cascade.
Our patient had psychomotor and developmental delay, infantile seizures, abnormal coronary vessels, persistent foramen ovale, bifid uvula and cleft palate. Sequencing of the genes involved in the RAS-RAF-MAPK signaling pathway (PTPN11, SOS1, RAF1, KRAS, HRAS, BRAF, MEK1, MEK2) did not show a pathogenic mutation. We thus applied whole genome array technology (Affymetrix 6.0) to search for submicroscopic chromosomal rearrangements. SNP-chip analysis revealed a de novo 1.17 Mb microdeletion of chromosome 1q42.11 encompassing 12 genes (FBXO28, CNIH4, DEGS1, NVL, WDR26, CNIH3, DNAH14, LBR, ENAH, SRP9, LEFTY3, and EPHX1). The gene product of one of the deleted genes, WDR26, has been described as a negative regulator of the RAS-MAPK signaling cascade. Thus, we sequenced WDR26 in a cohort of 200 patients with NCFC features and tested negative for mutations in known causative genes. No sequence alteration with pathogenic relevance could be detected. We are currently investigating SRP9 and ENAH – encoding two other interactors of the MAPK cascade – in the same cohort and will, if sequence alterations are not being detected in these patients, expand our investigations to other candidate genes within the deleted region.

Carbon nanoparticle (CNP) induced transient pulmonary inflammation in mice: indications of IL1B, IL18 and VEGF cascade in maintenance of homeostasis

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Background:
A massive release of combustion products and the revolution in nanotechnology increasingly exposes mankind to carbon nanoparticles (CNP). The proinflammatory response caused by pulmonary CNP deposition is a key event for its adverse health effects. Epidemiological studies suggest individuals with favourable lung physiology to be at lower risk for particulate matter related respiratory disease. In this study mice with robust lung physiology were exposed to moderately toxic CNP with the aim to study the elicited pulmonary inflammation and its resolution.
Methods:
5, 20, and 50µg CNP were intratracheally instilled in C3H/HeJ mice to identify the optimal dose for subsequent time course studies. Histology, broncheoalveolar lavage (BAL) analysis and a panel of 62 protein markers were used to assess lung inflammation.
Results:
Particle challenge resulted in a dose response pattern 24h after instillation, with 5µg dose representing the 'no effect level' as reflected by polymorphonuclear leucocyte (PMN) influx, and BAL/lung concentrations of pro-inflammatory cytokines and proteins. Accordingly 20 µg dose was used to assess the inflammatory time course after 3 and 7days. At day 3 BAL PMN counts were significantly decreased compared to 24h, and by day 7 complete resolution was observed. Protein markers related to acute and time course response were identified in lung and BAL. On day 7, concentrations of 20 proteins including FGF2, VEGF, IL1B, IL18 and EDN1 were still significantly elevated. These factors are involved in a closely regulated molecular cascade with IL1B/IL18 (upstream) and FGF2/EDN1/VEGF at the downstream. BAL-protein concentration and histological analysis revealed no evidence of tissue injury at 20µg dose.
Conclusion:
Considering the role of VEGF, FGF2 and EDN1 in lung development and morphogenesis together with a lack of any tissue damage we suggest activation of a protective machinery to counter the CNP challenge and to preserve homeostasis.

MVD013 a mouse model of inherited polycythaemia

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MVD013, a mutant mouse line showing a dominantly inherited polycytemia phenotype in the peripheral blood cell count, was established within the Munich ENU mouse mutagenesis project by the Clinical Chemistry Screen. Additionally, gastrointestinal tumors are found at the ileum and caecum of almost all aged mutant mice. SNP analysis showed the highest possibility of the mutation to be located on chromosome 5, and fine mapping revealed the c-KIT oncogene as the most promising candidate gene. KIT-mutations in humans are known to be associated with haematological, fertility and pigmentation disorders as well as gastro intestinal tumors (1). A comprehensive phenotypic characterization of this mouse line by the German Mouse Clinic (2) identified additional effects on the distribution of leucocyte subsets in peripheral blood and the cardiovascular system as well as gastro-intestinal fuction and pathology. Since MVD013 shows similar symptoms like human patients suffering from Polycythemia Vera (PV) or Gastrointestinal stromal tumors (GIST) it might represents a model that can be used to elucidate regulatory mechanisms that may play a role in the development of GISTs or polycytaemias in humans.

Miettinen M and Lasota J (2005): KIT (CD117): a review on expression in normal and neoplastic tissues, and mutations and their clinicopathologic correlation. Appl Immunohistochem Mol Morphol 13 (3): 205-220

Gailus-Durner et al. (2009): The German Mouse Clinic: a platform for systemic phenotype analysis of mouse models. Curr. Pharm Biotechnol 10 (2): 236-243

From Gene to Clinic: TMA based clinical validation of molecular markers in prostate cancer

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One of the most important challenges of molecular cancer research in the next years is the extensive clinical validation of molecular data using clinically relevant endpoints.
Current high-throughput screening techniques using DNA arrays have identified hundreds of new candidate biomarkers for diagnosis and risk prediction of several cancers. Large-scale analysis of clinical cancer specimens is a key prerequisite for the validation of these genes. We have constructed a tissue microarray from more than 3,000 prostate cancers with full histo-pathological and clinical long term follow-up data, and analyzed expression and gen copy number patterns of 16 different candidate markers for their ability to predict prostate cancer progression and patient prognosis. The best candidates were used to extend established clinical prediction tools (nomograms) that were based on non-molecular data only, like prostate specific antigene (PSA), clinical stage and histological grading (Gleason grade). Using this approach, we could identify ANXA3 as an independent marker, which was capable to increase the accuracy of the clinical nomogram and therefore fulfills criteria of a novel prognostic prostate cancer marker. This approach of integrating large-scale clinical and molecular variables may provide a new paradigm for the use of molecular profiling to predict clinical outcome in prostate cancer.

Identification of Chromosomal Changes in Primary Prostate Carcinoma Patients by Multicolor Fluorescence in situ Hybridization

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Conventional cytogenetic analysis often reveals complex changes with unresolved abnormalities, such as marker chromosomes, and is limited in detecting small and cryptic aberrations. Multicolor fluorescence in situ hybridization (M-FISH) is a powerful method to elucidate complex aberrations and is able to identify novel, recurrent genetic abnormalities.

In this study, 37 biopsies of patients with prostate carcinoma were recruited for their investigation by conventional cytogenetic analysis and M-FISH. For the culturing of the prostate carcinoma cells, we used a two-step short-term culture technique that combined a mechanical and an enzymatic disaggregation, followed by growth in a serum-free medium. Chromosomal analyses could be performed in 23 out 37 cultures (62%). The modal chromosome number was diploid in 10 patients and tetradiploid in 13 patients. Two cases presented two concomitant clones showing a tetraploid male karyotype and a diploid male karyotype with a trisomie 7 in one case and with a trisomy 20 in the other case. Structural chromosomal aberrations were not detected in any of the patients analyzed.

Our study shows that numerical abnormalities are the most recurrent alterations in prostate carcinoma patients. However, cryptic imbalances targeting small chromosomal regions could remain beyond the limits of detection of M-FISH and should be analyzed with more sensitive techniques.

Identification of a Novel Effector Gene for Multiple Human Cancers using Recombinant Cell Line Technology

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Expression profiling commonly delivers more or less comprehensive sets of genes, which are up- or downregulated in association with cancer stage or prognosis. Downstream of such approaches, a functional validation in well-defined in vitro systems represents the essential first step in order to discern true effector genes from merely correlated genes and to explore the underlying regulatory networks.
We started from a set of 35 genes, which were selected based on differential expression in brain and other cancer types or based on belonging to the group of yet uncharacterized protein kinases. We used a recombination based system that was developed by us and that allows for the facilitated construction of isogenic stable cell lines with quantitatively regulable expression of target genes. An initial proliferation/viability screen in the cell line U373-MG identified a tyrosine kinase (tk33) with so far unknown function, which exerted strong killing effects on brain cancer cells upon activation.
Consecutive studies of tk33 in different cancer cell lines showed, that its activation and inhibition affects cancer cell viability. Furthermore, we found tk33 upregulated in various cancer types. Overexpression of a putative kinase-deficient variant induced apoptosis to a higher extent than the full-length tk33, presumably because of a dominant negative effect.
Based on our results, tk33 might play an important role in multiple cancer types and its further characterization is essential in order to gain more insight into its involvement in cancer signalling.

Recurrent regions of deletions involving the ERG-TMPRSS2 fusion gene and the PTEN tumor suppressor gene in prostate cancer

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DNA copy number profiling experiments using classical comparative genomic hybridization (CGH) suggested that high-level amplifications and deletions are less frequent in prostate cancer as compared to other solid tumors. It is possible, however, that small deletions and amplifications exist, which cannot be easily identified in low-resolution CGH experiments. In order to screen for such small alterations, we have analyzed fresh-frozen tissue samples from 77 prostate cancer samples (n=41 Gleason =4+3, n=36 Gleason =3+4; median PSA 15,4 (range 1.9-100); n=67 localized cancers, n=10 metastatic cancers) on Affymetrix Genome-Wide Human SNP Array 6.0.

Gross chromosomal alterations were found in the majority of cases (70/77, 91%), including loss of 8p (15, 6%) and gain of 8q (11, 7%) as the most frequent findings. High-level amplifications were not detected. Small regions of deletions were found most frequently at chromosome 21q22.2-21q22.3, and 10q23. A detailed analysis of the breakpoints showed that the interstitial deletion at 21q22.2-q22.3 corresponds to the formation of a fusion transcript between the ERG gene, located at the centromeric edge of the deleted region and the TMPRSS2 gene at the telomeric edge of the deletion. The minimally deleted region at chromosome 10q23 encompassed the gene locus of the PTEN tumor suppressor gene.

These data demonstrate that high resolution SNP array data can help to identify currently undetected fusion genes and tumor suppressor genes in prostate cancer.

An evaluation of the genetic-matched pair study design using genome-wide SNP data from the European population

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The effects of incomplete Mendelian randomization in population-based gene–disease association studies can potentially be alleviated by genetic matching . We therefore evaluated the genetic-matched pair study design on the basis of genome-wide SNP data from >2400 individuals recruited at multiple sites across Europe. We tried to derive a subset of markers that would allow identification of the best overall matching (BOM) partner for a given individual, based on the pair-wise identity-by-state (IBS) for the subset alone. However, this approach yielded a notable improvement in prediction accuracy only by the first 20 markers selected, while accuracy increased proportionally to the marker number thereafter. Furthermore, in the majority of cases (76.0%), a given individual and his/her BOM partner originated from different recruitment sites. A second marker set, specifically selected for ancestry sensitivity using singular value decomposition, performed even more poorly and was no more capable of predicting the BOM than randomly chosen subsets. In conclusion, the utility of the genetic-matched pair study design depends, at least in Europe, critically on the availability of comprehensive genotype information.

Bioinformatic challenges of sequencing whole-genome and MHC haplotypes with NGS technology

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The human Major Histocompatibility Complex (MHC) is one of the most important genetic regions in relation to common disease. In our project entitled 'MHC Haplotype Sequencing: An Integrated Approach to Common Disease' use is made of a unique haploid reference resource (HRR) consisting of fosmid libraries from 100 individuals from a representative German population cohort, PopGen (http://www.popgen.de/). Fosmids are pooled together in pools of 5000 cfu or superpools containing 15,000 cfu. Next Generation Sequencing (NGS) is conducted using an Applied Biosystems SOLiD V3 sequencer. The aim of the project is to assemble MHC and genomic haplotype coverage from the discontinuous coverage resulting from pool and super-pool sequencing.

A common focus in NGS technology is on genomic re-sequencing by assembling sequence against a reference. In the first instance the Human Genome Project generated a reference sequence for the MHC from a patchwork of libraries in diverse institutions, which was later replaced by that from a single haplotype (HLA homozygous PGF cell line; the longest single-haplotype assembly in the current reference genome). There are currently seven alternative full length MHC single haplotype sequences available (Genome Reference Consortium), together with several other, shorter, haplotype specific sequences. These are all potential alternative references for use in assembly.

Against this background we address the bioinformatic challenges of the current project which include:

- fosmid mapping
- fosmid read coverage detection
- fosmid based tiling and phasing
- haplotype sorting
- structural variation discovery
- improved fosmid based short read mapping

MHC Haplotype Sequencing: An Integrated Approach to Common Disease

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The human major histocompatibility complex (MHC) is recognized as the most important genetic region in relation to common diseases including inflammatory, infectious and autoimmune diseases as well as transplant medicine. Major national and international genome research networks have demonstrated associations between the MHC region (~ 4 MB) and numerous disease phenotypes of interest. To move from the regions of association to the causative variants, the highly complex nature of the MHC needs to be resolved. This requires sequencing individual MHC molecular haplotypes directly and completely, both at the population level and in diseases.

To this aim we have established key resources and technologies: 1) A unique 'Haploid Reference Resource' of 100 human fosmid libraries from a representative German population cohort (PopGen; 200 haploid genomes), and 2) a SOLiD next generation sequencing (NGS) and data analysis pipeline that includes project-specific modules of fosmid detection and sub-genome matching. To generate MHC haplotype sequence information, the following, complementary, approaches have been taken in parallel:
1) Classically, SNP-based mapping and isolation of MHC haplotype-informative fosmids from the clone pools, separate assembly of two MHC fosmid tiling paths, subsequent NGS of those; 2) hybridisation-/PCR-based enrichment of MHC sequences from the fosmid clone pools by use of microarrays coupled with NGS; specifically, HybSelect (Febit), NimbleGen Sequence Capture (Roche), SureSelect RNA baits (Agilent), a microfluidic chip (Fluidigm) and a microdroplet technology (RainDance) are being applied and comparatively evaluated; 3) direct NGS of the haploid clone pools (5000 or 15000 fosmids/pool, respectively). Combination of these approaches allows assembly of first MHC haplotype sequences. Moreover, our lines of research and production provide a valuable platform to tackle highly variable genomic regions and generate haploid sequences on a broader scale.

Reliability of functional imaging paradigms for multicenter studies

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As the findings from functional magnetic resonance imaging (fMRI) studies gain soaring influence on current neuroscientific research and beyond, the issue of the data reliability becomes more and more urgent and important. This particularly applies for imaging genetics studies which are focussing on the impact of genetic risk variants for psychiatric disorders on brain function. Different ways of calculating reliability of fMRI data are reported in the literature, e.g. using voxel-wise intra-class correlation coefficient (ICC) analysis (Specht et al., 2003; Caceres et al., 2009).
For quality control implemented in an NGFNplus project we scanned 36 healthy subjects twice with an interval of 14 days in between. Data were acquired on three study sites (Mannheim, Bonn and Berlin) on a Siemens Trio whole-body 3T MR system.
Reliability in terms of ICCs were calculated and compared for the three study sites as for the different cognitive and emotional paradigms applied (associative memory, working memory, emotion regulation, inhibition, reward, theory of mind). Reliability results will be presented by focussing on the task-related brain regions compared to other areas and effectiveness of the approach will be discussed in detail. We conclude that reliability analyses are beneficial and essential in order to ensure data quality and therefore to improve the significance of findings in fMRI research.

References:
Caceres A, Hall DL, Zelaya FO, Williams SC, Mehta MA. Measuring fMRI reliability with the intra-class correlation coefficient. Neuroimage. 2009;45(3):758-68.
Specht K, Willmes K, Shah NJ, Jäncke L. Assessment of reliability in functional imaging studies. J Magn Reson Imaging. 2003;17(4):463-71

Mapping determinants of human hippocampal gene expression by genome-wide association

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Recent studies have stressed the extent to which gene expression varies within and between populations, and have shown that allele-specific expression is relatively common among non-imprinted autosomal genes. Much of the genetic component of human phenotypic diversity has been proposed to be the result of cis-acting influences on gene expression. In the present study, we aim to identify genetic factors that influence gene expression in the human hippocampus. For systematic mapping of determinants for hippocampal gene expression, we isolated genomic DNA and RNA of hippocampus samples taken from the Bonn tissue bank and performed a genome-wide association study (GWAS) and gene expression (GEX) analysis. Each individual DNA sample is genotyped with >600,000 SNPs. Individual gene expression levels for more than 99,9% of all known human genes (approx. 25,000 annotated RefSeq and UniGene genes) are interrogated with microarrays containing more than 48,000 probes. Gene expression levels are then systematically correlated with individual genotype information. In addition we are establishing lymphocyte cell assays to characterize regulatory elements via Chromatin immune-precipitation (ChIP). One important application of our findings will be the interpretation of SNP association findings for brain phenotypes, in particular neuropsychiatric disorders.

Identifying disease associated SNPs using biological pathway knowledge

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Genome wide SNP measurements by high density arrays result in measurements for up to millions of SNPs. Checking each SNP independently for an association with a disease is difficult due to the problem of multiple testing. Including information on biologically relevant groupings of SNPs and testing only within these groups can reduce the problem of multiple testing dramatically.
One way to incorporate biological knowledge is testing SNPs in groups predefined by biological knowledge. The Global test was developed for gene expression data and can be applied to categorical variables. It tests the influence of expression strength of genes on disease status based on pathway groupings of genes.
We implemented a method analysing data from genome wide association screens in the context of pathways. After mapping the SNPs to genes the globaltest is conducted based on the SNP status instead of gene expression values.
A data set on major depressive disorder comparing the genome wide SNP status of 597 affected and 1223 control samples was analysed using pathway definitions from several databases.

The neuronal transporter gene SLC6A15 confers risk to major depression

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Major depression is one of the most prevalent psychiatric disorders and a leading cause of loss in work productivity. A combination of genetic and environmental risk factors likely contributes to this disorder. We present data from a genome-wide association study revealing a neuron-specific neutral amino acid transporter (SLC6A15) as a novel candidate gene for major depression. Risk allele carrier status and chronic stress are associated with a downregulation of the expression of this gene. Decreased expression of this transporter appears to be associated with reduced structural integrity of the hippocampus, a brain region implicated in the susceptibility for major depression. Our convergent data from human genetics, expression studies, animal models and brain imaging suggest a novel pathophysiologic mechanism for major depression that may be accessible to drug targeting.

REFINED PHENOTYPING REGARDING GABAA RECEPTOR GENES AND THEIR IMPACT ON BIPOLAR DISORDER

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There is increasing evidence that refinement of phenotype of complex psychiatric disorders can produce stronger association signals. Craddock et al.[1] recently reported that the index association signal obtained using a set of GABAA receptor genes in bipolar disorder was enriched and became an order of magnitude stronger in a much smaller subsample of DSM IV bipolar patients who had been assigned a refined RDC diagnosis of "schizoaffective disorder, bipolar (SABP)." In order to replicate this finding, we re-diagnosed n=682 DSM IV bipolar patients included in our previous GWA study according to RDC criteria based on OPCRIT data (RDC-SABP, n=352, and, RDC-nonSABP, n=330). We applied a gene-based approach using all available SNPs (n=362) in the 19 GABAA receptor genes. While no association with a DSM IV diagnosis of bipolar disorder was observed, significant association (p=8.8x10-3) was obtained when only SABP patients were included. The genes making a major contribution to these results were GABRB2 (p=1.9x10-4), GABRG2 (p=1.2x10-2) and GABRA1 (p=1.7x10-2). The strongest SNP association was obtained for rs7714930 (p=1.1x10-5) within GABRB2. Our results thus replicate the association findings for RDC-SABP with GABAA receptor genes, and show that the refining of major phenotypes may be a prerequisite to the identification of genetic risk factors for complex psychiatric disorders.
[1] Craddock et.al. Mol Psychiatry, 2008 Jul 1. [Epub ahead of print]

Genome-wide association study identifies a novel susceptibility gene for bipolar disorder and schizophrenia, neurocan (NCAN)

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We conducted a genome-wide association study (GWAS) and follow-up in bipolar disorder (BD; 6,024 patients and controls). Genetic variation in neurocan (NCAN) showed genome-wide significant association with BD (P = 3.02E-8) and improved to P = 5.74E-10 after including a schizophrenia sample (1,332 patients and controls). After ANK3, which was recently reported by an independent study and is also nominally associated in our BD data set, this is the second genome-wide significant finding that has emerged from a GWAS of BD. Further, our finding supports previous findings that BD and schizophrenia share a portion of their genetic background. NCAN expression in mice co-localized with brain areas where neuro-imaging studies identified abnormalities in BD patients that may be indicative of disturbances in key neuronal circuits.

Association of Genetic Variation in KCNQ1 with Type 2 Diabetes in the KORA Surveys

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Recently, a significant association between variants of the KCNQ1 gene and type 2 diabetes has been reported. Replication of polygenetic disease associations has often revealed false positive findings and overestimated effects in initial reports in the past. Therefore, this study aimed to replicate the association of the variant rs2237895, which was originally found in an Asian population and assesses its effect on type 2 diabetes in a case-control study of 2.697 KORA participants (1230 cases/ 1467 controls). We further extended our analyses to the glycemic traits in a population based sub sample of 1092 fasting KORA participants. Results of our analyses confirmed the minor C allele as risk variant for type 2 diabetes (OR=1.12 [1.00,1.25]; p=0.025). This association could be ascribed to the association in men (OR=1.16 [1.00,1.35]; p=0.022). Assessing effects for metabolic parameters, significantly increased fasting glucose levels were found for the C allele of rs2237895 (beta estimate=1.39 mg/dl; p=0.01) in men, supporting affection of pancreatic beta cell function by variants in KCNQ1 leading to type 2 diabetes by means of impaired insulin secretion. However, mechanisms remain unclear and absence of the effect in women has to be elucidated in further studies. Finally, our results underline a role of KCNQ1 variants in type 2 diabetes and related traits in Caucasians.

A putative functional threonine79-methionine (Thr79Met) substitution in the ileal fatty acid binding protein (FABP6) also known as gastrotropin might be a risk marker of Type 2 Diabetes in obese persons

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The ileal fatty acid binding protein (FABP6) is known to be involved in enterohepatic bile acid metabolism. We have previously found a significant association between the rare allele of the FABP6 Thr79Met (rs1130435 C>T) polymorphism and lower type 2 diabetes risk in a small case–control study (192 cases and 384 controls) embedded in the large EPIC-Potsdam cohort. A priori functional implication of the amino acid change was gained from in silico analysis. In this study, we analysed an independent nested case–cohort including 543 incident type 2 diabetes cases from the EPIC-Potsdam cohort and a case–control study including 939 type 2 diabetes cases from KORA to confirm the association with type 2 diabetes and performed association analyses with quantitative disease-related measures in 2112 non-diabetic individuals. Homozygosity for the Met-allele – present in 16% of the individuals studied - was associated with a 24% lower risk of type 2 diabetes (95% confidence interval: 8% to 38%) (EPIC-Potsdam: 30%, P = 0.04; KORA: 21%, P = 0.06) if adjusted for age, sex, body mass index (BMI), and waist circumference. The homozygous rare variant showed a significant interaction (P = 0.006) with BMI. Risk estimates in different categories (BMI<25, BMI 25–30, and BMI>30 kg/m2) showed an association exclusively in obese (BMI>30 kg/m2) individuals (combined risk ratio: 0.62, 95% CI 0.45–0.86). In non-diabetic individuals from the general adult population, no significant associations were observed with plasma total cholesterol, LDL-, and HDL-cholesterol, triglyceride, insulin and glucose concentration. In summary, we found evidence that the putative functional Thr79Met substitution of FABP6 confers a protective effect on type 2 diabetes in obese individuals from to independent German cohort studies.

Sequencing out Effects of Resveratrol on Chromatin

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Sirtuin 1 (Sirt1), a NAD+-dependent deacetylase, has been implicated in the delay of aging and the prevention of diabetes, obesity, and some cancers. Resveratrol, a compound identified in red wine, was shown to activate Sirt1 and thus modulate metabolic responses by deacetylating transcriptional factors. Sirt1 is also known for its role in the deacetylation of histone proteins at specific lysine residues. To date, the effect of resveratrol on histone modifications through Sirt1 and corresponding gene transcription has yet to be determined. We used the approach of chromatin immunoprecipitation followed by sequencing (ChIP-Seq) to compare binding patterns between untreated and resveratrol-treated murine skeletal muscle cells. Although resveratrol treatment induces global deacetylation of histone H1 on lysine 26 as shown by western blotting, it unexpectedly generates dense acetylation patterns of this histone modification on the exons of important metabolic sensors, namely Sirt1, Pparg, and Lxra. This histone acetylation pattern is, amongst others, associated with upregulated Sirt1, Pparg, and Lxra gene expression. Resveratrol also induces binding of other histone proteins and Sirt1 on various genes involved in glycolysis, gluconeogenesis, and fat oxidation. Our results demonstrate how dietary therapeutic treatment is likely to influence the expression of metabolically active genes through histone modifications. We furthermore show concepts for the mechanisms of resveratrol treatment, which reveal new pathways for treating metabolic diseases and slowing aging processes.

Identification of the transcription factor Zfp69/ZNF642 as a candidate gene for obesity-associated diabetes

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Background: Type 2 diabetes is associated with obesity and results from a combination of adipogenic and diabetogenic alleles. Here we report the identification of a candidate gene for the diabetogenic effect of the mouse QTL Nidd/SJL. The susceptible allele was contributed by the lean SJL inbred mouse strain in an outcross population with New Zealand Obese (NZO) mice.
Methods: In order to identify the responsible gene variant for Nidd/SJL we generated recombinant congenic mice carrying different fragments of the QTL on the diabetes resistant C57BL/6J background. To test these fragments for their ability to induce diabetes we performed reporter crosses with NZO mice.
Results: The reporter crosses of recombinant congenic mice led to the identification of a critical region of chromosome 4 that contained ten genes. Sequencing, qRT-PCR, and RACE-PCR identified a remarkable variant of Zfp69 encoding zinc finger domain transcription factor 69. In NZO and C57BL/6J, a IAPLTR1a retrotransposon in intron 3 disrupted the gene by formation of a truncated mRNA that lacked the coding sequence for the Krüppel-associated box (KRAB) and Znf-C2H2 domains of Zfp69, whereas the diabetogenic SJL allele generated a functional mRNA. When transferred to the B6.V-Lepob background, the diabetogenic Zfp69SJL allele produced hyperglycaemia, reduced gonadal fat pads, and increased plasma and liver triglycerides. mRNA levels of the human orthologue of Zfp69, ZNF642, were significantly increased in adipose tissue from patients with type 2 diabetes.
Conclusion: The data indicate that Zfp69 is the most likely candidate gene for the diabetogenic effect of Nidd/SJL, and that retrotransposon IAPLTR1a contributes substantially to the genetic heterogeneity of inbred mouse strains. Expression of Zfp69/ZNF642 in adipose tissue may play an important role in the pathogenesis of human type 2 diabetes.

Characterization of a putative obesity gene which affects nutrient-dependent insulin secretion

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Background: Obesity represents one of the most important contemporary health problems and has a polygenic basis. In order to identify novel obesity genes we employed a strategy that combined two genome-wide screens in two different species (siRNA-mediated mutagenesis in C. elegans and a QTL analysis in a polygenic obesity mouse model, the New Zealand obese mouse). For one putative obesity gene there is evidence from human studies that a splice site mutation of the human ortholog is associated with hyperinsulinism of infancy. Furthermore, an in-vitro study indicated that this gene is a novel regulator of insulin secretion.
Methods: In order to corroborate this assumption, we deleted the gene with a gene trap approach and characterized the corresponding mouse model with respect to body weight development and glucose homeostasis under standard and high-fat conditions.
Results: Under standard-diet conditions, no differences in body weight and body composition were detected between knockout and control mice. However, on a high-fat diet knockout mice displayed a reduced body weight and a reduced fat mass. Randomly measured blood glucose and insulin levels were similar in knockout and wild-type mice under standard and high-fat diet conditions. But after fasting and a 2 hour refeeding period, blood glucose levels were significantly lower in knockout mice presumably due to higher insulin levels. Interestingly, insulin secretion of knockout mice was only increased in response to a fat bolus, whereas glucose-induced insulin secretion was impaired in knockout mice.
Conclusion: We identified a gene which is involved in diet-dependent alterations of the body weight and in the regulation of nutrient-dependent insulin secretion.

On the association of copy number variation with early onset extreme obesity - Preliminary results of a genome-wide association study

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Obesity is a major health problem. Although heritability of obesity is substantial, elucidation of the genetic mechanisms predisposing to obesity is ongoing. With the advent of genome-wide association studies (GWAS), several genomic regions and candidate genes have been found to contribute genetic variants for obesity. To date these genomic regions only explain a small proportion of the overall heritability of the obesity phenotype. Thus, copy number variations (CNVs) have been recently suggested to contribute to the dissection of complex human diseases.

We analysed two different GWAS samples to address the question of the effect of CNVs on early onset extreme obesity. The first GWAS sample consists of 424 case-parent trios, i.e. comprising an extremely obese child or adolescent and both biological parents. The second GWAS sample consists of 453 extremely obese children and adolescents and 435 normal weight or lean controls. All individuals were genotyped with the Genome-Wide Human SNP Array 6.0 from Affymetrix.

First, we analysed both GWAS samples exploratively with the aid of their copy number variation calls aiming for the identification of common copy number variation regions (CNVRs). Subsequently, in order to avoid inaccury due to the choice of the calling algorithm and the reference sample, we based our statistical analysis in the estimated CNVRs on the fluorescent intensities as a measure for copy number. For association analysis in the family-based sample we applied the CNV FBAT methodology proposed by Ionita-Laza et al. (2008). The association between obesity and raw copy number signals in the case-control sample was analysed using logistic regression and generalized linear models.

Two CNVRs that have been reported to be associated with adult BMI in a Chinese and German sample, could be confirmed by at least one of our analyses. In addition, we detected new CNVRs which we currently followed-up in independent samples with similar ascertainment schemes.

In silico Identification of Candidate-SNPs for Causal Variants

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SNP-chips which are used in genome-wide association studies (GWAS) permit the genotyping of up to 4 million single nucleotide polymorphisms (SNPs) in one experiment. Up to now more than 1000 human SNPs were found to be statistically significantly associated with a phenotype (e.g. a disease). It is often difficult to identify the gene, which affects a specific phenotype. However, mutations within the causal gene can be in linkage disequilibrium (LD) with surrounding polymorphisms. SNPs found to be significant in association studies thus represent a valuable source of information about the location of the causal gene. Thus, for a deeper understanding of the molecular mechanisms of genetic diseases, identifying candidate SNPs in high LD with significant SNPs is indispensable.

We developed the software tool CandiSNPer to facilitate the identification of candidate SNPs for causal variants. CandiSNPer determines the HapMap-SNPs which are in LD with a given significant SNP from a GWAS. This defines a flanking region of LD around that SNP. Then CandiSNPer provides a list with functional annotation, association with genes, and LD-values for all SNPs with reference IDs (rsIDs) found in the flanking region. The result is graphically visualized. Due to the much higher abundance of rsSNPs as compared to HapMap-SNPs, candidate SNPs for causal variants are more likely to be discovered than by analyzing HapMap SNPs alone.

Summarizing, CandiSNPer allows extending the range of interesting SNPs by identifying SNPs in linkage disequilibrium with significant SNPs from GWAS. CandiSNPer helps to efficiently assess SNPs based upon their functional class and the genes that are located in their neighborhood. CandiSNPer is available free of charge at
http://www2.hu-berlin.de/wikizbnutztier/software/CandiSNPer.

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Association of MC4R variants with insulin levels but not with lipid metabolism markers in German overweight children and adolescents

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Background: Association with obesity and insulin resistance was recently reported for two single nucleotide polymorphisms (SNPs) rs17782313 and rs12970134 located downstream of the melanocortin-4 receptor gene (MC4R) in two independent genome-wide association studies (GWAS).
Methods: In up to 889 German overweight and obese children and adolescents we analysed association of rs17782313 and rs12970134 with obesity related-traits and fasting blood parameters indicative of insulin resistance and lipid metabolism such as HDL/LDL cholesterol and insulin levels (13 endophenotypes in total).
Results: We confirmed the strong association of both MC4R SNPs with overweight and early onset obesity (e.g., rs17782313 odds ratio (OR)CT=1.40 95% confidence interval (CI) [1.16…1.70], ORCC =1.96 95% CI [1.35…2.88]; nominal p=0.0003; adjusted for 14 statistical tests p=0.0042) in comparison to 442 healthy underweight controls. In addition, we observed higher insulin levels for carriers of the reported risk-alleles (e.g., for rs12970134 ß(per risk allele)=0.033; 95% CI [0.005;0.060]; nominal p=0.020; adjusted p=0.28) in comparison to obese subjects homozygous for the non-risk alleles while we observed no evidence for association of the SNPs to the other endophenotypes such as fasting lipid levels.
Conclusions: SNPs rs12979134 and rs17782313 near the MC4R confer a risk for early onset obesity; additionally they have an influence on insulin levels.

Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS)

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The polycystic ovary syndrome (PCOS), a common endocrine disorder in women of child-bearing age, mainly characterised by chronic anovulation and hyperandrogenism, is often associated with insulin resistance (IR) and obesity. Its etiology and the role of IR and obesity in PCOS are not fully understood. We examined the influence of validated genetic variants conferring susceptibility to obesity and/or type 2 diabetes mellitus (T2DM) on metabolic and PCOS-specific traits in patients with PCOS.

Methods: We conducted an association study in 386 patients with PCOS (defined by the Rotterdam-criteria) using single nucleotide polymorphisms (SNPs) in or in proximity to the fat mass and obesity associated gene (FTO), insulin-induced gene-2 (INSIG2), transcription factor 7-like 2 gene (TCF7L2) and melanocortin 4 receptor gene (MC4R). To compare the effect of FTO obesity risk alleles on BMI in patients with PCOS to unselected females of the same age range we genotyped 1,971 females from the population-based KORA-S4 study (Kooperative Gesundheitsforschung im Raum Augsburg, Survey 4).

Results: The FTO risk allele was associated with IR traits and measures of increased body weight. In addition, the TCF7L2 SNP was associated with body weight traits. For the SNPs in the vicinity of INSIG2 and MC4R and for the other examined phenotypes there was no evidence for an association. In PCOS the observed per risk allele effect of FTO intron 1 SNP rs9939609 on BMI was +1.56 kg/m2, whereas it was +0.46 kg/m2 in females of the same age range from the general population as shown previously.

Conclusion: The stronger effect on body weight of the FTO SNP in PCOS might well have implications for the aetiology of the disease.

Sirtuins as targets for studying Molecular Mechanisms in Obesity

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The sirtuins are a class of proteins, which act by removing acetyl groups from proteins in the presence of NAD+. They are responsible for a number of key cellular regulation processes in yeast and in mammals. Sirtuin activation has been demonstrated to influence the activity of metabolic sensors including subsequent effects on gene expression and post-translational modifications of proteins. In rodents, the natural product resveratrol has been shown to mimic dietary energy/calorie restriction by activating SIRT1 and extending the healthy lifespan of animals fed a high-fat diet. In a nutshell, SIRT1 is a promising target in the discovery of dietary therapeutics, which may affect efficiently the enzymatic activity of this protein.

The bioavailability of resveratrol and other activators of SIRT1 is fairly low in humans. To identify alternative sirtuin modulators we set up a high-throughput screening assay using MALDI-TOF mass spectrometry. The activity of SIRT1 was observed by detecting the molecular mass of the increasing amounts of deacetylated peptides. We screened a library of ~5500 natural products for activators and inhibitors and discovered 1 novel activator and 15 new inhibitor molecules. Using direct mass spectrometry detection of SIRT1 substrate peptides we could circumvent artefacts of fluorescence-based assays. In the next step we performed quantitative mass spectrometry analysis of the interaction of SIRT1 with these molecules.

In order to enable structural analysis of the human SIRT1 and its interacting small molecules, we have prepared affinity tagged E. coli expression clones. We designed five truncated constructs, and successfully cloned and purified them by small scale protein expression and affinity purification. Soluble expressed proteins are currently being prepared in large scale for crystallisation trials and protein-ligand binding reactions. These reactions are analysed by thermal shift assay (TSA) and isothermal titration calorimetry (ITC).

A DNA-hypermethylation polymorphism in the POMC gene is associated with childhood obesity and affects a P300 binding site

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Mutations in the POMC and MC4R gene, that are embedded in the leptin-melanocortin signalling cascade of central body weight regulation, lead to severe early onset obesity. We tested the hypothesis, if in addition to classical genetic defects also epigenetic alterations of the POMC gene locus might be associated with human obesity. We investigated the DNA methylation pattern of the POMC CpG islands by bisulfite-sequencing. Functional studies of P300 binding were performed with ChIP analysis and qPCR.? First we described the methylation pattern of the POMC gene locus which revealed a distinct methylation pattern at the 5´ POMC CpG island in human peripheral blood cells (PBC), which was conserved in mice PBC. In the 3´ CpG island, we obtained in human PBC a sharp boundary of DNA methylation with a hypermethylated intron 2 and a completely hypomethylated exon 3. Both DNA methylation patterns were reproducible in microdissected ß-MSH positive human postmortem brain samples and PBC-DNA extracted from newborn screening cards, indicating a stable pattern, which is present directly after birth. We further analyzed the DNA methylation in PBC of 81 obese childhood patients and 54 normal weight individuals with two independent bisulfite-based methods. We found a significant hypermethylation of 10 CpG positions at the intron2-exon3 intersection in obese patients ($p<0,001$). Moreover at the first CpG position within the exon3, which is significantly hypermethylated in the obese cohort, we confirmed a binding site of the histone acetyltransferase P300 by ChIP analysis and reveal a reduced P300 binding capacity in hypermethylated, obese patients in qPCR analysis. Therefore we describe for the first time a DNA-hypermethylation polymorphism that is significantly associated with childhood obesity. The hypermethylation polymorphism is located within a P300 binding region of the 3´POMC CpG island suggesting an effect on chromatin formation and altered POMC gene expression.

Computational analysis of evolution and function of CAG/glutamine repeats

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Several neurodegenerative conditions such as Huntington's disease are caused by the expansion of CAG trinucleotide repeats or polyglutamine (polyQ) stretches over specific length thresholds. During disease progression the affected proteins form intracellular aggregates and recruit additional proteins into these inclusions. So far, neither the wild type function of the non-expanded repeats nor the pathomechanism following the length extension are fully understood. For example, it is still under debate if function and toxicity of the repeats are properties at the RNA or at the amino acid level.
At first, we provide evidence that the genomic distribution of CAG repeats has been shaped by positive evolutionary selection. Besides being enriched in coding exons the repeats preferentially locate to Untranslated Regions suggesting a function already at RNA level. These observations are complemented by a phylogenetic analysis revealing several loci where polyQ repeats appear independently at different time points during evolution in groups of orthologous proteins.
For the repeat function at amino acid level we discuss a potential involvement in protein-protein interactions. Based on the observation that polyQ containing proteins have a significantly higher amount of interaction partners compared to other proteins we investigated a role for polyQ as a protein interaction domain by assessing the deviation of the polyQ protein-protein interaction network from random topology. We observed several enriched domains among them polyQ itself in the interaction network surrounding the polyQ protein set. Together these findings suggest a dual role for CAG/glutamine repeats both at the RNA and at the amino acid level.

Modulation of Protein Complex Composition and Function involved in Neurodegenerative Diseases

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Many efforts have been made to increase our understanding of the cause, progression and mechanism leading to neurodegenerative disorders like Alzheimer disease, Parkinson disease or amyotrophic lateral sclerosis. Whereas most of these diseases are sporadic and of unknown cause, a series of different gene mutations have been identified to cause familial variants of several neurodegenerative disorders. For the purpose of modelling disease related signalling pathways neurodegenerative disease relevant protein-protein interactions have to be identified and characterised. In addition we want to discover the consequence of genetic aberrations on a biochemical as well as on a functional level.
To achieve this goal we have adapted an inducible Flp-In T-REx HEK 293 cell culture system to the GATEWAY high-throughput cloning system for transfection and efficient generation of stable cell lines. So far we produced 24 stable cell lines expressing a C- or N-terminal TAP-fusion protein for SOD1, SNCA, APP, ATXN1, PARK2, PARK7, PSEN1 and HTT (wild type and mutant forms). Native protein complexes are isolated by tandem affinity purification (TAP) and analysed via mass spectrometry. Functional assays are further employed to study the morphological and cellular traits as a consequence of neuronal disease related protein overexpression of wild type and mutant variants. In the future, disease-relevant cues such as cellular stress or co-expression of upstream regulatory kinases will be applied in our cellular system to investigate the consequence of such modulation on protein complex composition and function.

Human genetic factors in tuberculosis: candidate genes and a genome-wide association study

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Human genetic factors in infectious diseases are of crucial interest as they may point to critical metabolic pathways and suggest intervention targets.
In a case-control design, 2010 patients with sputum/culture positive tuberculosis and 2346 healthy controls from Ghana, West Africa, were identified and DNA samples were subjected to candidate genotyping. Likewise, mycobacterial isolates were characterized with respect to mycobacterial species and genotypes (spoligotyping, IS6110 typing).
While the MCP-1 promoter variant -362C provided increased protection against pulmonary tuberculosis (OR 0.81) caused by all mycobacterial species studied, differential susceptibility patterns were observed for (i) the exonic ALOX variant 760A which was associated with susceptibility to infections caused by the mycobacterial lineage Mycobacterium africanum West-African 2 (OR 1.70), (ii) the promoter variant -261 of IRGM that was found associated with protection from mycobacteria of the M. tuberculosis EUAM lineage (OR 0.66) and (iii) the MBL G57E variant that was associated with protection from the species M. africanum. The latter association was confirmed functionally by demonstrating stronger binding of MBL to M. africanum than to M. tuberculosis.
The results of a joint analysis of genome-wide association studies (Affymetrix SNP array 6.0 and 500 K) with inclusion of additional population controls and a case-control cohort from The Gambia revealed strong associations with markers on chromosome 18 (P = 10-9) and on chromosome 2 (P = 10-7).
The underlying causative variants are currently subject of further analyses and will hopefully lead to the identification of new targets for intervention.

Quantifying the effect of sequence variation on regulatory interactions

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The increasing amount of sequence data provides new opportunities and challenges to derive mechanistic models that can link sequence variations to phenotypic diversity. Here we introduce a new computational framework to suggest possible consequences of sequence variations on regulatory networks. Our method, called sTRAP, analyses variations in the DNA sequence and predicts quantitative changes to the binding strength of any transcription factor for which there is a binding model.
We have tested the method against a set of known associations between SNPs and their regulatory consequences. Our predictions are robust with respect to different parameters and model assumptions. Importantly we set an objective and quantifiable benchmark against which future improvements can be compared. Given the good performance of our method, we developed a publicly available tool which can serve as an important starting point for routine analysis of disease-associated sequence regions.

A comprehensive analysis of FCER1 genes reveals epistatic effects of FCER1A variants on total IgE levels and eczema risk

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Background: In a population-based genome-wide association scan, we recently identified the gene encoding the alpha chain of the high affinity IgE receptor (FCER1A) as new susceptibility locus for serum IgE levels. Previous studies suggested linkage and association of IgE and related phenotypes to the gene encoding the beta chain of the high-affinity IgE receptor (FCRER1B). So far, the gene encoding the gamma chain of the high-affinity IgE receptor (FCRER1G) has not been investigated with respect to allergic traits. Null alleles of the gene encoding the key epidermal protein filaggrin (FLG) are the most highly significant and widely replicated genetic risk factor for eczema.
Methodology/Principal Findings: We investigated the association of FCER1A, FCER1B and FCER1G with IgE and atopy in a large population-based cohort and tested for interaction between these loci. In addition, we investigated a potential interaction between FLG mutations and FCER1A variants in a large collection of eczema cases and population controls. For analysis of epistasis we used the Model-Based Multifactor Dimensionality Reduction (MB-MDR) method. Three strongly correlated FCER1A polymorphisms were significantly associated with total and specific IgE levels. No associations were seen for FCER1B and FCER1G variants. Strong locus-specific epistasis was observed between the two FCER1A variants rs2251746 and rs16842010 (p_value <0.0001). After adjustment for FLG effects, a significant epistatic effect of the two FCER1A variants rs10489854 and rs2511211 was detected.
Conclusions/Significance: The findings indicate locus-specific interplay effects of FCER1A variants on total IgE levels and eczema, and highlight the significance of epistasis in complex or quantitative traits.

No evidence for a role of COL29A1 variants in eczema and atopic diseases

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Background:
Based on a recent positional cloning approach, it has been suggested that COL29A1, which is located in a linkage region on chromosome 3q21 and encodes the collagen VI 5 chain, represents a major susceptibility gene for eczema (atopic dermatitis).
Objectives:
To investigate the association of COL29A1 variants with eczema and related traits. To specify the expression pattern of COL29A1 in the skin.
Methods:
A set of 7 COL29A1 single nucleotide polymorphisms (SNPs) reported to be associated with eczema as well as the most common filaggrin (FLG) mutations were genotyped in four independent samples: a collection of 1687 German eczema cases and 2387 population controls, an eczema cohort of 274 German parent-offspring trios, a cross-sectional population of German children (n = 3099), and the Swedish population-based birth cohort BAMSE (n=2033). In situ hybridization using a COL29A1-specific cRNA-probe was carried out on skin biopsies of eczema patients and controls.
Results:
No associations were seen for any of the COL29A1 SNPs or haplotypes with eczema in any of the samples. The absence of associations was confirmed by equivalence tests. In the German cross-sectional cohort, no associations with asthma, rhinitis or specific IgE were observed. There was no abnormal cellular distribution pattern of COL29A1 expression in the epidermis of eczema patients.
Conclusions:
Previously reported associations of COL29A1 with eczema were not replicated in this study. Our results suggest that COL29A1 is unlikely to contain polymorphic loci that have a major impact on eczema or atopy susceptibility.

Genome-wide association study for ulcerative colitis demonstrate novel associations on chromosomes 22 and 7

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Several clinical and pathological features distinguish ulcerative colitis (UC) from Crohn's disease (CD). Whereas there have been more than 30 susceptibility loci identified for CD in recent genome-wide studies, there is still a strong need for further identification of disease specific susceptibility loci in ulcerative colitis (UC). Most of the UC susceptibility genes reported so far have been found to be also involved in Crohn's disease, and the involved pathways can therefore be attributed to more general functions with its disturbance contributing to both subtypes of inflammatory bowel disease (IBD). Due to the lower relative sibling risk in UC (6-9) as compared with Crohn's disease (5-35), susceptibility genes in UC have indeed proven to be harder to detect than in Crohn's disease. We performed a genome-wide association analysis of 666,009 high-quality single nucleotide polymorphisms (SNP) using the Affymetrix® Genome-wide human SNP array 6.0 and typing 1043 UC patients and 1703 healthy controls of German origin. According to the genomic inflation factor of 1.127, genetic heterogeneity was low. Novel UC specific associations outside of previously reported regions were visually inspected to exclude false positive associations. The 175 most strongly associated SNPs passing quality control were genotyped in additional panels of European descent totaling 3582 UC patients and 7131 healthy controls in the final combined analysis. Besides 28 others, the strongest new association signals were detected in regions on chromosome 22 and chromosome 7 with highly significant replication P-values (P=4.21×10-08 and P=8.81×10-11, respectively). Both loci encode biologically interesting candidates with regard to UC pathogenesis. The lack of associations in the previously reported Crohn's disease GWAS meta-analysis indicates that these associations are most probable to be UC-specific.

CNVineta: A data mining tool for large case-control copy number variation (CNV) data sets.

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Many recent research findings suggest that copy number variation plays a major role in genetic variability. After the first release of the human genome, the variation types which got highest attention were the less complex variations like SNPs and low-complexity tandem repeats. But today we see that the copy number variations like deletions and duplications can span megabases and affect more bases per individual than SNPs do.
To screen our Affymetrix Genome-Wide Human SNP Array 6.0 data with more than 7000 samples (derived from NGFN GWAS initiative) we developed the R package CNVineta for rapid data visualization and mining. CNVineta allows the usage of the CNV analysis software of your choice, for example Affymetrix Power Tools or quantiSNP. After running data conversion scripts and executables, thousands of SNP chip samples can be rapidly processed. With CNVineta, additional quality controls can -
be applied, .fam and .cnv.map files for PLINK are generated, and the PLINK analysis results for rare and common CNV screening are integrated for data mining purposes. For both rare and common CNVs custom screening rules can be defined and graphical overviews are subsequently generated. These overviews comprise segmental CNV data, raw data LRR and BAF plots, and heat maps. Triggers for additional detailed table output can be set. Region overviews show the predicted segments of all samples within their genetic context, i.e. localization of SNP and copy number probe sets of the SNP chip and gene annotations. The next screening step in CNVineta is the visualization of the raw data in heat maps. These heat maps can be generated cohort-wide or reduced to the affected and a random selection of flanking samples only. Heat map sorting functionality helps to evaluate common CNV regions more efficient. Finally all raw data and segment table can be processed by existing/custom R functions.

FOXO3A, a confirmed longevity gene in humans

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Human longevity is influenced by multiple genetic and environmental factors. The genetic component to this phenotype is estimated at 25-32%. Until recently, only variation in the apolipoprotein E gene (APOE) was found to be consistently associated with longevity in diverse populations. Although numerous case-control candidate gene studies have been performed and associations of the longevity phenotype with biologically plausible genes have been described, results from these experiments have proven difficult to validate. In September 2008, Willcox et al. published a study describing the association of variation in the forkhead box O3A (FOXO3A) gene with human longevity (Proc Natl Acad Sci USA 105:13987–13992). FOXO3A is an evolutionarily conserved key regulator of the insulin-IGF1 signalling pathway. The important role of this "master regulator" in diverse biological pathways including stress resistance, apoptosis, immune regulation and inflammation renders FOXO3A a very convincing candidate. However, the Willcox' results were tentative as they had not been replicated in an independent population. Therefore, we have investigated 16 known FOXO3A single nucleotide polymorphisms (SNPs) in our collection of 1762 German centenarians/nonagenarians and younger controls. Our results provide conclusive evidence that polymorphisms in this gene are indeed associated with the ability to attain exceptional old age (Proc Natl Acad Sci USA 106: 2700-2705). Furthermore, we observed that the FOXO3A association was considerably stronger in centenarians than in nonagenarians, highlighting the importance of the oldest old for genetic longevity research. However, so far no functional SNPs in FOXO3A have been reported that are known to influence the longevity phenotype. A strategy for the identification of causative variants in the gene (rare and/or common) will be presented.

A GENOME-WIDE APPROACH FOR IDENTIFICATION OF SHARED GENETIC SUSCEPTIBILITY LOCI BETWEEN PRIMARY SCLEROSING CHOLANGITIS AND INFLAMMATORY BOWEL DISEASE

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Background and aims. Genome-wide association studies (GWAS) and subsequent meta-analysis projects have been successful in identifying novel genetic susceptibility factors for several autoimmune and inflammatory diseases. Although many examples exist that different disorders share common genetic risk loci, systematic and genome-wide approaches are lacking. We therefore started a joint genome-wide analysis of various immune-related diseases to detect and characterize new disease susceptibility alleles that are relevant for more than one of these diseases.
Methods. We have recently completed GWAS for various complex diseases. More than 5000 controls and 7000 cases for 11 distinct diseases were genotyped on different Affymetrix or Illumina arrays. In the first overlap analysis, three distinct, however clinically related, diseases of the intestinal tract were included: Of the 7000 cases, 1121 German ulcerative colitis (UC), 480 German Crohn's disease (CD), and 406 German primary sclerosing cholangitis (PSC) patients were genotyped using whole-genome arrays. Standard quality thresholds were applied to all data sets before and after imputation with MACH leaving >1.6 Mio. SNPs for inclusion in the analysis.
Results. Two separate analysis strategies are planned: 1) Selection of SNPs that were significant in PSC, but not in the UC GWAS. 2) Selection of SNPs that were significant in PSC and UC. For a fast-track replication study, we already selected 191 SNPs for replication: 41 for CD-UC, 133 for UC-PSC, 32 for CD-PSC, and 18 for CD-UC-PSC. First results will be presented at the conference.
Conclusions. The project takes a systematic, genome-wide approach by studying an overlay of GWAS data sets from different diseases in clinically relevant combinations. A large-scale replication of the identified loci as well as subsequent resequencing of replicated disease loci will identify the broad spectrum of variation and will yield lists of potentially causative variants.

Genome-wide Association Study for Psoriasis

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Psoriasis is a chronic immune-mediated and hyperproliferative disorder of the skin, nails and joints that affects up to 3% of the Caucasian population. The first identified and now well-confirmed susceptibility locus is PSORS1 on chromosome 6 in the MHC-class1-region. In several studies polymorphisms in IL12B and IL23R have been found to be associated with psoriasis susceptibility. Other loci with newly confirmed association, identified by a recent genome wide association study (GAIN consortium), encode IL23A, a gene involved in IL-23 signaling, TNIP1 and TNFAIP3, two genes that regulate NF-?B signaling and IL4 and IL13 that are involved in the modulation of Th2 immune responses.
To identify additional psoriasis susceptibility loci, we successfully genotyped 561,466 SNPs in 472 psoriasis cases and 1146 controls of German ancestry. We followed up the 145 most significant SNPs in 2746 psoriasis cases and 4140 controls. This replication cohort consists of 681 cases and 1824 controls from Germany, 1303 cases and 1322 controls from USA (GAIN, in silico replication) and 762 cases and 994 controls from Canada (Genizon, in silico replication). In order to combine the results of all 3 cohorts and to include effect size parameters, we performed a Meta-analysis using METAL. Our results provide support for the association of psoriasis and the confirmed locus IL12B (Meta-analysis $P < 6.5 \times 10\text{-}14$) as well as for a novel gene1 (Meta-analysis replication $P < 6.3 \times 10\text{-}06$), which encodes a protein involved in regulating responses to cytokines by members of the NF-?B transcription factor family. For two other SNPs, located 30 kb downstream of gene2 and 25 kb upstream of gene3 respectively, marginal evidence for association was observed but did not remain significant after correcting for multiple testing by Bonferroni. Our results confirm the importance of NF-?B and IL23 signaling in auto-immune disorders like psoriasis. All three novel loci will be revealed during the conference.

Improving data and quality management for high-throughput analyses

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The identification of genetic factors involved in complex diseases is based on the establishment of reliable links between phenotypic and genotypic data of the patients. Therefore the access to large resources of genotyping and sequencing capacities as well as systematic data processing and analysis under strong quality guidelines are fundamental. Within the NGFN-subproject T1 we have established a number of high quality standards and methods for a cost- and time efficient follow-up of novel candidate genes identified by the current GWAS and candidate gene approaches. This includes the implementation of an automated allele concordance analysis system into the SNPlex technology, the upgrades of established systems (LIMS, SOLiD) and the establishment of a SNP-chip databank. In addition, a highly automated state-of-the-art quality control and analytical workflow has been established for GWAS. In brief, quality controls comprise per sample (e.g. call rate, deviation from expected heterozygosity, duplicate/related/"outlier" samples, sex-check) as well as per SNP analyses (e.g. call rate, allele frequencies, deviation from Hardy-Weinberg-equilibrium in controls, automated cluster plot generation). We have also set up an automated plotting pipeline that allows to visually distinguish between likely technical artifacts and genuine association signals. Another QC and plotting pipeline has been set up for genome-wide CNV data derived from Affymetrix 5.0 or 6.0 arrays.

Genome-wide CNV screening in monozygotic discordant twin pairs for inflammatory bowel disease identifies tissue- and disease-specific candidate loci

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Genetic predisposition to Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of inflammatory bowel disease (IBD), has not been resolved completely yet. So far only 10% of the cumulative genetic variance can be explained by the identified susceptibility loci, so much of the heritability remains unkown. Recently, several studies have highlighted the contribution of copy number variations (CNVs) and other structural rearrangements to the pathogenesis of IBD (e.g. deletion upstream of autophagy gene IRGM associated with CD) and other complex inflammatory diseases (e.g. deletion in LCE gene cluster associated with psoriasis). Interestingly, there is evidence that discordant monozygotic twins display different CNV profiles due to somatic mosaicism, leading to the assumption that disease-susceptibility loci might also be found in these de novo copy number variant regions
In order to identify CNVs that evolved through somatic mosaicism and predispose to IBD, we have conducted a comprehensive screen of CNVs in 9 monozygotic twins by means of high-resolution, genome-wide array-comparative genomic hybridization (array-CGH) using the Nimblegen 2.1M array with a detection resolution of ~5 - 10kb. Our samples comprised 3 twin pairs discordant for UC, 3 pairs discordant for CD and 3 healthy twins. For each twin pair, genomic DNA from both blood and bowel biopsy of the disease individual was compared to blood DNA of its healthy co-twin. To this end, we have identified 31 chromosomal aberrations representing candidate CNVs in the disease-affected tissue of interest. We are currently validating the chip-results using an independent technology and will then aim for a replication in independent monozygotic discordant twin pairs as well as in available large case-control collections for IBD.

UNIFYING CANDIDATE GENE AND GWAS APROACHES IN ASTHMA

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Background A genome wide association study (GWAS) for childhood asthma identified a novel major susceptibility locus on chromosome 17q21 harboring the ORMDL3 gene, but the role of previous asthma candidate genes was not specifically analyzed in this GWAS.
Methods We systematically identified 98 SNPs in 19 candidate genes previously associated with asthma in =3 independent study populations. We re-genotyped 50 SNPs in these genes not covered by GWAS in 703 asthmatics and 658 reference children. Genotyping data were compared to imputation data derived from Illumina 330K HapMap chip genotyping. Results were combined to analyze 615 SNPs covering all 19 candidate gene loci.
Results Genotyped polymorphisms in ADAM33, GSTP1 and VDR showed effects with p-values <0.0025. Combining genotyping and imputation, polymorphisms in DPP10, EDN1, IL12B, IL13, IL1RN, IL4, IL4R and TNF showed associations at a significance level between p=0.05 and p=0.0025.
Conclusions These data indicate that (a) GWAS coverage is insufficient for many asthma candidate genes, (b) imputation based on these data is reliable but incomplete, and (c) SNPs in three previously identified asthma candidate genes replicate in our GWAS population with significance after correction for multiple testing in 19 genes.

Functional Annotation of Candidate Disease Genes: Molecular Mechanisms regulating NOD-like Receptor Activity

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NOD-like receptors (NLRs) represent cytosolic sensor proteins for intracellular danger signals such as ´microbe – associated molecular patterns´ (MAMPs, e.g. peptidoglycan/PG, muramyl-dipeptide/MDP) or ´danger-associated molecular patterns (DAMPs, e.g. low K+, urate crystals). Elicitor-induced oligomerization and refolding of NLR proteins typically results in activation of proinflammatory signalling cascades (MAPKs, NF-kappaB, caspase-1). The general importance of concerted NLR activation for innate immune responses is emphasized by identification of several NLRs as genetic susceptibility factors for chronic inflammatory disorders (e.g. Crohn disease, Atopy) and general fever syndromes (e.g. Muckle-Wells Syndrome, Familial Mediterranean Fever). Here we show that NLR activity is tightly regulated at different cellular levels. Using the MDP sensor protein and Crohn disease risk factor NOD2 as example, we show how alternative splicing affects specific signalling mechanisms such as activation of transcription factor NF-kappaB. Furthermore, we demonstrate the involvement of reactive oxygen species (ROS) in NLR activity acting both as modifiers and direct effectors of anti-bacterial defense mechanisms. Finally, the role of specific protein/protein interactions for control of NLR activation is depicted and an RNAi-based functional screening approach for identification of NLR-regulating gene networks is described.

Characterization of Functional Crosstalk between NOD-like Receptors (NLRs) and the Autophagy Pathway

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Autophagy represents an intracellular proteolytic degradation system in which cytoplasmic components (e.g. protein aggregates, organelles, cytosolic bacteria) are enclosed by a spheric double-membrane structure and directed to the lysosomal compartiment for hydrolytic degradation. Recently, we have identified autophagy gene ATG16L1 as a novel genetic susceptibility factor for Crohn Disease (CD), a chronic relapsing-remitting inflammatory disorder of the bowels. By now several studies have pointed to pivotal role of autophagy induction and execution for etiopathology of chronic inflammatory disorders. Interestingly, genetic data display locus interaction between ATG16L1 and the well-characterized CD risk gene NOD2. NOD2 encodes for a cytosolic pattern recognition receptor that recognizes intracellular presence of muramyl dipeptide (MDP) derived from bacterial cell walls. The functional basis for this genetic interaction still remains to be elucidated.
In this study we introduce a novel cell-culture-based assay that uses fluorescence signals originating from protein-fragment complementation events to quantify induction of autophagy. This approach is used to study the role of NOD2 and other pattern recognition receptors for initiation of autophagy in the context of bacterial cytoinvasion (Listeria infection model). The established methodology will help to decipher the complex interplay between cellular machineries that mediate recognition of and defence against cytoinvasive pathogens.

Both copy number and sequence variations determine expression of human DEFB4

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Copy number variations (CNVs) were found to contribute massively to the variability of genomes. Around 12% of the human genome are covered by CNVs (SNPs ~0.3%). One of the best studied CNV region is the ß-defensin cluster (DEFB) on 8p23.1. Individual DEFB copy numbers (CN) between 2 and 12 were found. Whereas low copy numbers predispose for Crohn disease, high numbers predispose for psoriasis. A further level of complexity is represented by sequence variations between copies (for instance as single nucleotide variations - MSVs) which are suggested to determine phenotypes, as SNPs do. Little is known about the relation of DEFB CN and MSVs to the expression of ß-defensin genes. To address this we analyzed the DEFB4 expression in B-lymphoblastoid cell lines (LCL) and primary keratinocytes (NHEK) before and after stimulation with LPS, TNF-a and IFN-?. Moreover, we quantified a cMSV in DEFB4 in DNA and mRNA as a marker for allele-specific expression (ASE) and resequenced a region of ~2 kb upstream of DEFB4 in LCLs. We found a strong correlation of DEFB CN and DEFB4 expression in 16 LCLs, although several LCLs with very different CNs exhibit similar expression levels. Quantification of the MSV revealed unequal expression of the MSV-alleles with consistently lower expression of one allele. In NHEKs but not in LCLs DEFB4 expression is induced by cytokines. Co-stimulation of NHEKs with TNF-a/IFN-? leads to a synergistic increase in total DEFB4 expression and, interestingly, suppresses ASE. By analysis of the DEFB4 promoter region we noticed remarkably high sequence variability (~1 MSV/41 bp). For the reliable quantification of DEFB CN we established an improved Paralogous Ratio Test (PRT) suited for high throughput applications.

Deciphering NOD2-dependent signalling by systematic RNA-interference

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The intracellular NOD-like receptors (NLRs) comprise a family of cytosolic proteins that have been implicated as ancient cellular sentinels mediating protective immune responses elicited by intracellular pathogens or endogenous danger signals. Genetic variants in NLR genes have been associated with complex chronic inflammatory barrier diseases (e.g. Crohn disease, bronchial asthma). NOD2 is a well characterized member of the NLR-family and variants of NOD2 are associated with Crohn disease. It is triggered by muramyl dipeptide which is a common cell wall brick of gram-positive as well as gram-negative bacteria. Some members of the NOD2-dependent signalling pathway are already described. The aim of this approach is to unravel the signalling pathway of NOD2 using RNA interference (RNAi). For this purpose, we established a RNAi screening method that can be used to identify further components of the NOD2-dependent signalling pathway. 24 out of 274 initial candidate genes passed through two independent verifications. RIPK2 and RELA, both important components of this pathway, are among these candidates and likewise BIRC4 (XIAP) which was connected to NOD2 lately. A new bioinformatic evaluation method supports the quality of the screening results and can be used for similar high-throughput experiments. Finally, preliminary data using IL-8 release as an alternative readout substantiate the importance of the new identified candidates for the NOD2-dependent signalling pathway. The list of candidate genes opens a wide spectrum of feasible experiments and will sustain the understanding of NOD2-dependent signaling.

Statistical analysis of the structural impact of cancer-associated mutations

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One central aim of cancer research is to understand the functional consequences of mutations that are causally linked to oncogenesis. In this study, we apply bioinformatical methods to structurally characterize somatic mutations commonly occurring in selected tumor types. From the results we obtain, we can validate general trends for the impact of cancer mutations on protein structures. The goal is to develop a high-throughput method that can easily be applied to large sets of genes and mutations. In the long run, we hope to be able to predict the functional impact on oncogenesis of newly identified mutations based on the localization in the protein structure.

For a set of ~200 mutations (selected from the COSMIC database) we analyse their localization with respect to surface accessibility, co-localization, proximity to functional sites and the effect on protein stability. For statistical significance we compare the observed results to two control sets, a null model of random mutations and a set of mutations frequently occurring in healthy populations (SNPs).

Preliminary results show substantial differences between oncogenes and tumor suppressors. While mutations of oncogenes tend to occur at the protein surface and directly affect sites important for protein function, mutations of tumor suppressors tend to affect protein stability rather than functional sites. In both cases, mutations are significantly more clustered when compared to random mutations or SNPs.

Nicotinic acetyl cholin receptor beta 2 subunit gene (CHRNB2) variants are associated with altered levels of glutamate and glutamine (Glx) in cingulate cortex and cerebral white matter of schizophrenic patients

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Introduction: The gene encoding the beta 2 subunit of neuronal nicotinic acetylcholine receptors (CHRNB2) has been linked to schizophrenia (De Luca et al 2006) and nicotine dependence (Greenbaum et al 2006). Nicotine dependence is exceedingly frequent in schizophrenic patients and therefore a common pathophysiology has been proposed (Mobascher and Winterer 2008). Of note, nicotinic acetylcholine receptors are involved in cerebral glutamate metabolism (Lambe et al 2003) and accumulating evidence indicates a glutamatergic dysfunction in schizophrenia (Lang et al 2007). The aim of this study was to show an association of CHRNB2 gene single nucleotide polymorphisms (SNPs) with cerebral glutamate and glutamine levels in schizophrenic patients.
Methods: A sample of 52 schizophrenic patients was measured using proton magnetic resonance spectroscopy at 3 Tesla. Voxels were positioned in the anterior cingulate cortex (ACC), the posterior cingulate cortex (PCC) and right frontal white matter (WM). Glutamate levels were assessed as ratio of glutamate+glutamine/creatine (Glx/Cre). Investigated CHRNB2 SNPs were rs1127317, rs2072659, and rs2072660.
Results: Carriers of the C/C genotype of SNP rs2072659 showed lower Glx/Cre values in the ACC, the PCC, and the WM than carriers of the other genotypes ($p=0.041$, $p=0.023$, and $p=0.065$, respectively). Similarly, carriers of the SNP rs2072660 C/C genotype showed lower values of Glx/Cre in the ACC and WM than carriers of the other genotypes ($p=0.046$ and $p=0.024$, respectively). For SNP rs1127313 the impact of genotype on Glx/Cre in the WM showed a statistical trend ($p=0.079$).
Conclusion: The results indicate an impact of genetic variations of the nicotine system on the glutamate status. Effects were observed in cerebral regions with special relevance for the pathophysiology of schizophrenia. The observed association may contribute to the understanding of a suggested common pathobiological pathway of schizophrenia and smoking addiction.

A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium

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The number and volume of cells in the blood are the most commonly measured blood parameters in clinical practice and impact on a wide range of disorders including cancer, cardiovascular, metabolic, infectious and immune disorders. We carried out a meta-analysis of eight different hematological parameters in 13,943 samples from six European population-based studies. We identified 22 genetic loci associated with hematological parameters, including six associated with red blood cell traits (e.g. the HBS1L-MYB region on 6q23-q24 (rs9402686, P = 7.42 x 10-42), the C282Y amino acid change in HFE at 6p21.3 (rs1800562, P = 1.44 x 10-23) and TMPRSS6 (P = 9.5 x 10-10)), one associated with white blood cell parameters (GSDM1/ORMDL3, P = 9.41 x 10-9) and 15 associated with platelet counts and volume (e.g. BET1L, P = 1.3 x 10-14; BCL2L7P1/BAK1, P = 3.7 x 10-10 and JMJD1C P = 3.30 x 10-21). The loci identify known and novel regulators of hematopoiesis, underscoring the high level of connectivity in stem cell fate determination. We further used whole-genome expression data in stem-cell derived blood lineages to study patterns of gene expression, and identified the first platelet eQTL near BET1L (P = 3.1 x 10-5). We finally tested associations with coronary artery disease in 9,479 cases and 10,527 controls and identified one long-range haplotype at 12q24 carrying 10 SNPs associated with this disease (OR = 1.152, 95% CI 1.104-1.202, P = 7.05x10-11 for the best SNP). We show that the haplotype, which also contains known risk loci for type 1 diabetes, hypertension and celiac disease, underlies a locus of high disease pleiotropy. Using evolutionary analyses we show that the haplotype has been spread by a selective sweep which began ~4,300 years ago and was specific to European and nearby populations.

Reference: Soranzo et al. A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. Nature Genetics, in press.

Meta-Analysis of 28,141 Individuals Identifies Common Variants within Five New Loci that Influence Uric Acid Concentrations

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Elevated serum uric acid levels cause gout and are a risk factor for cardiovascular disease and diabetes. To investigate the polygenetic basis of serum uric acid levels, we conducted a meta-analysis of genome-wide association scans from 14 studies totalling 28,141 participants of European descent, resulting in identification of 954 SNPs distributed across nine loci that exceeded the threshold of genome-wide significance, five of which are novel. Overall, the common variants associated with serum uric acid levels fall in the following nine regions: SLC2A9 (p=5.2 x 10-201), ABCG2 (p=3.1 x 10-26), SLC17A1 (p=3.0 x 10-14), SLC22A11 (p=6.7 x 10-14), SLC22A12 (p=2.0 x 10-9), SLC16A9 (p=1.1 x 10-8), GCKR (p=1.4 x 10-9), LRRC16A (p=8.5 x 10-9) and near PDZK1 (p=2.68 x 10-9). Identified variants were analyzed for gender differences. We found that the minor allele for rs734553 in SLC2A9 has greater influence in lowering uric acid levels in women and the minor allele of rs2231142 in ABCG2 elevates uric acid levels more strongly in men compared to women. To further characterize the identified variants, we have analyzed their association with a panel of metabolites. rs12356193 within SLC16A9, was associated with DL-carnitine (p=4.0 x 10-26) and propionyl-L-carnitine (p=5.0 x 10-8) concentrations, which in turn were associated with serum UA levels (p=1.4 x 10-57 and p=8.1 x 10-54, respectively), forming a triangle between SNP, metabolites and UA levels. Taken together, these associations highlight additional pathways that are important in the regulation of serum uric acid levels and point towards novel potential targets for pharmacological intervention to prevent or treat hyperuricemia. In addition, these findings strongly support the hypothesis that transport proteins are key in regulating serum uric acid levels.

A Genome-wide Association Study Identifies GLT6D1 as a Susceptibility Locus for Periodontitis

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Periodontitis is a widespread, complex, inflammatory disease of the mouth, which results in a loss of gingival tissue and alveolar bone, with aggressive periodontitis (AgP) as its most severe form. To identify genetic risk factors for periodontitis, we conducted a genome-wide association study (GWAS) in German AgP patients. We found AgP to be strongly associated with the intronic SNP rs1537415, which is located in the glycosyltransferase gene GLT6D1. We replicated the association in a panel of Dutch generalized and localized AgP patients. In the combined analysis including 1,919 subjects, rs1537415 reached a significance level of P = 8.02 x 10-7, OR = 1.49 (95% CI 1.27-1.75). The disease associated rare G allele of rs1537415 showed an enrichment of 10% in periodontitis cases (48.4% in comparison with 38.8% in controls). Fine-mapping and a haplotype analysis indicated that rs1537415 showed the strongest association signal. Sequencing identified no further associated variant. Tissue specific expression analysis of GLT6D1 indicated high transcript levels in the leukocytes, the gingiva and testis. Analysis of potential transcription factor binding sites at this locus predicted a significant reduction of GATA-3 binding affinity, and an electrophoretic mobility assay indicated a T cell specific reduction of protein binding for the G allele. Overexpression of GATA-3 in HEK293 cells resulted in allele specific binding of GATA-3, indicating the identity of GATA-3 as the binding protein. The identified association of GLT6D1 with AgP implicates this locus as an important susceptibility factor, and GATA-3 as a potential signaling component in the pathophysiology of periodontitis.

Long-term follow-up in extended myocardial infarction families demonstrate high risk for cardiovascular events

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The aim of our study was to identify genetic factors that contribute to the development of coronary artery disease (CAD) and myocardial infarction (MI). It was assumed that genetic burden is higher in extended MI families with young age at first manifestation. Methods: We recruited 24 extended families (513 individuals) with at least 5 members in the same generation being affected with severe CAD, i.e. index patient suffered from MI<60 years and other family members were affected by MI, percutaneous coronary intervention (PCI) or coronary bypass (ACB) below 70 years. Affection status was classified to “affected” (known CAD) or “non-affected” (no CAD and at least 10 years older age than the youngest age of onset of CAD within this family). All other individuals were classified “unknown”. Standardized questionnaire were taken at baseline, in 2-, 5-and 10year follow-up.
Results: At baseline, 23.8% of individuals were judged as “affected”. The mean age of first CAD manifestation was 49.7 and 44.8 years in female and male patients, respectively. On average 7.8 years after recruitment, a follow-up examination was performed, during which 19 family members changed their phenotype status from “unknown” to “affected”. We observed 5 MIs, 4 ACBs, and 12 PCIs. 106 family members (20.7%) turned from “unknown” to “unaffected” status due to age criteria (63.2% female, mean age at follow-up 72.3 years). In total, 44 deaths occurred (8.6%), of which 24 (54.5%) were caused by CAD. Most coronary deaths occurred in the “affected” group (81.8%), but were highly prevalent in the “unknown” and “unaffected” group (in 18.2%). Conclusions: After a mean of follow-up of 7.8 years, 27.5% of extended MI family members were affected by severe CAD. 19 family members, who were “unknown” at recruitment, suffered from MI, PCI or ACB (4.9%). Therefore, the risk for severe CAD and cardiac death is considerably higher in families carrying a strong genetic burden as compared to the general population.

Detecting subgroups and multilocus effects by means of clustering algorithms on genotype data

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In the past decade, high-throughput gene chip technologies have yielded enormous amounts of genotype data. The majority of genome-wide association (GWA) studies have only investigated single genetic risk factors, e.g. SNPs. However, single SNP effects might not reveal the more complex genetics underlying multifactorial traits. The lack of studies investigating interaction effects might result from the computational challenge ofdealing with large amounts of genotype data. Thus the need for appropriate data mining approaches is obvious.

Clustering algorithms can be a useful approach for detecting patterns in large data sets. The SNPs that are characteristic for these patterns might give clues about the underlying genetic interactions specific for subgroups of individuals, thus allowing for the identification of interacting loci without having to search through the complete data set. Here, we suggest a novel clustering algorithm which forms clusters or bubbles around pairs of individuals by allocating further individuals to a bubble according to their genetic similarity. We evaluate the effectiveness of the new algorithm on simulated GWA data.

The Normal and Tumour spectrum of Copy Number Variation: Copy Number alterations correlate with changes in gene expression in tumour transcriptome

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Copy number variations (CNVs) have been widely observed in normal humans and in tumor genomes, and are increasingly implicated in common disease and in cancer progression. Massively parallel sequencing allows powerful, unbiased genome-wide interrogation of CNVs. We sequenced genomic DNA from matched tumor/ normal samples of three patients with tongue/tonsillar cancer using the SOLiDTM System. Using a modified version of the SegSeq algorithm and controlling the false discovery rate, we compared the numbers of sequence reads from tumor samples to those from normal samples in 100kb windows; we identified at least 300 significant copy number changes (ranging from 1 kb to 71,000 kb and from 0 to 9 copies) per genome. We performed SOLiD sequencing of the whole transcriptome of the tumor and normal samples, and examined the correlation between genomic copy number variation and changes in gene expression between tumor and normal samples. We found a significantly positive correlation (0.76) between CNV and gene expression in a patient. In parallel, we developed an algorithm to detect copy number variation in a single sample. We calculate coverage in variable-sized genomic windows that are selected to contain a constant number of mappable positions. Within these windows, we normalize coverage based on predicted mappability and GC content. We then use a Hidden Markov Model for segmentation, and we apply empirically derived filters to the contiguous segments to call copy number variants. Of CNVs above 5kb, 89% are in the Toronto CNV database, suggesting a very high true positive rate. When we compare to an orthogonal data set, we detect 72% of predicted CNVs above 2kb in the same sample. These results are robust even at 5x coverage. Hypothesis-free genome-wide CNV detection at low coverage opens the way for CNV genotyping of many samples to elucidate the role of CNVs in complex disease.

Genotype-Phenotype Correlation in the 2q31.1 contiguous gene syndrome – eight new patients

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Microdeletions of the 2q31.1 region are rare, and in a few cases only, the affected genes have been determined molecularly. Here, we present the clinical and molecular findings of eight previously unreported patients with overlapping deletions in 2q31.1, including one mother-to-daughter transmission of an 1.4 Mb deletion.

The patients present with developmental delay (7/8), growth retardation (5/8), seizures (2/8) and a craniofacial dysmorphism consisting of microcephaly (4/8), short palpebral fissures (7/8), broad eyebrows with lateral flare (7/8), low-set ears with thickened helices and lobules (5/8), and micrognathia (6/8). Additional congenital anomalies were noted, including limb abnormalities (8/8), heart defects (3/8) genital anomalies (3/8) and craniosynostosis (1/8).

Seven of these microdeletions, ranging in size from 1.24 to 8.35 Mb, were identified by array CGH, one larger deletion (19.7 Mb) was detected by conventional karyotyping and further characterized by array CGH analysis. The smallest region of overlap in all eight patients spans at most 88 kb and includes only the WIPF1 gene. This gene codes for the WAS/WASL interacting protein family member 1. The protein binds to a region of the Wiskott-Aldrich syndrome (WAS) protein that is frequently mutated in the Wiskott-Aldrich syndrome (an X-linked recessive disorder) (MIM *602357). The patients described here do not present with clinical signs of the WAS and the deletion of this single gene does not allow explaining the phenotype in our patients.

It is likely that the deletion of different but overlapping sets of genes from 2q31 is responsible for the clinical variability of this contiguous gene syndrome. To further dissect the complex phenotype associated with deletions in 2q31, additional patients with overlapping phenotypes should be examined with array CGH. This should help to link particular phenotypes to specific genes, and add to our understanding of the underlying developmental processes.

Anti-inflammatory and Anti-cancer activities of Essential oils

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Natural products have been a continuous source of novel compounds for the treatment of numerous diseases. We evaluated the antitumor and the anti-inflammatory activity of 50 different plant extracts and their pure compounds in order to evaluate their potential and underlying mechanisms in pancreatic cancer cells. Humans pancreatic cancer cell lines (BxPC-3 and MiaPACA-2) were cultivated in RPMI-1640 or in DMEM medium supplemented. The human normal cell line (HPDE E6-E7) was maintained in the Keratinocyte-SFM. Approximately 5 x 103 growing cells were placed in each well of a 96-well plate with 200 µl of medium and were allowed to adhere overnight. Cells were then treated with each essential oil at concentration 100 ug/ml. The SRB cell proliferation Assay was used to assess cell viability. Anti-inflammatory activities were determined through measuring the inhibition of lipo-oxygenase enzyme and the level of PGE2. Moreover, the effect on nitric oxide release was also evaluated. Out of the 50 different plant extracts, 10 showed greater cytotoxic activities toward pancreatic cancer cell lines compared to the normal ones. Others showed powerful anti-inflammatory activities. Further analyses are required to establish the exact mechanisms modulating those effects.

A de novo t(1;8)(p35.2;p11.2) Translocation Associated with Severe Obesity and Hypogonadotropic Hypogonadism

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We report on a 18 years old female patient with severe obesity (BMI: 46 kg/m2) combined with hypogonadotropic hypogonadism resulting in amenorrhea. Cytogenetic analysis using high resolution GTG-banding revealed an apparently balanced de novo translocation described as t(1;8)(p35.2;p11.2). While the breakpoint region on chromosome 1 contains NR0B2 as a candidate gene for obesity, FGFR1 as a candidate for hyopogonadotropic hypogonadism is located within the breakpoint region on chromosome 8. Fluorescence in situ hybridization (FISH) analysis with BAC probes amongst others covering these two candidate genes showed signals on both derivative chromosomes indicating possible disruption of these genes, but exact breakpoint mapping is pending. NR0B2 (nuclear receptor subfamily 0, groub B, member 2) which is located on the predicted region on chromosome 1 is coding for an integrated factor of the cholesterol metabolism and was previously found to be mutated in patients with obesity. Mutations in FGFR1 (fibroblast growth factor receptor 1) were described as causative for autosomal dominant Kallmann syndrome of which hypogonadotropic hypogonadism is a major sign. Expression analysis of both genes in our patient is in progress.

Transcriptional regulation of three genes, C4, DAP3 and PKIB by antisense-oriented intronic endogenous retroviruses

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Human endogenous retroviruses (HERVs) are viruses that once infected ancestral germ line cells and became fixed in the genome, now making up about 8% of the human genome. A HERV located in intron 9 of the C4 gene on 6p21.33, HERV-K(C4), was shown to possess promoter activity in its 3'LTR in opposite direction to the encompassing gene, thus generating an antisense transcript with a suggested role in negatively regulating C4 gene expression. It was also demonstrated that LTR promoter activity is inhibited by interferon(IFN)-?-stimulation in a dose-dependent way. There are two C4 gene variants, a long form containing the HERV-K(C4) and a short form lacking the insertion. The C4 gene is part of the hypervariable RCCX region within the MHC and shows an extensive copy number variation. The repeated block in the RCCX has a length of about 26.5kb or 32.8kb in the presence of the short or long form of C4 respectively. Variation between copies of the HERV-K(C4) is low.
Performing BLAT searches against the 2009 human genome reference assembly we identified two HERV-K(C4)-related endogenous retroviruses located in antisense orientation within intron 1 of the DAP3 gene on 1q22 andwithin intron 2 of the PKIB gene on 6q22.31, respectively. DAP3 is an IFN-? inducible positive regulator of apoptosis and is over-expressed in numerous cancers. PKIB is an inhibitor of protein kinase C and G (PKC/PKG) and has recently been shown to be upregulated in prostate cancer.
We are conducting luciferase-reporter assays to test for promoter activity of HERV LTRs and assess the effect of cytokine stimulation. Furthermore, PCR-based techniques are used to detect putative antisense transcripts. Their effect on the expression level of the respective genes will be assessed by over-expression and strand-specific siRNA approaches. These data will allow us to elucidate the role of the presented HERVs in the regulation of their respective genes. Results will be presented here.

The CACNA1C polymorphism rs1006737 correlates with altered brain activation and impaired semantic verbal fluency in healthy individuals

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Background: Recent genetic studies found the A allele of the variant rs1006737 in the alpha 1C subunit of the L-type voltage-gated calcium channel (CACNA1C) gene to be overrepresented in patients suffering from bipolar disorder, schizophrenia or major depression. While the functions underlying the pathophysiology of these psychiatric disorders are yet unknown, impaired performance in verbal fluency tasks is an often replicated finding. We investigated the influence of the rs1006737 single nucleotide polymorphism (SNP) on verbal fluency and its neural correlates.
Methods: Brain activation was measured with functional magnetic resonance imaging (fMRI) during a semantic verbal fluency task in 63 healthy male individuals. They additionally performed more demanding verbal fluency tasks outside the scanner. All subjects were genotyped for CACNA1C rs1006737.
Results: For the behavioral measures outside the scanner, rs1006737genotype had an effect on semantic but not on lexical verbal fluency with decreased performance in risk-allele carriers. In the fMRI experiment, while there were no differences in behavioural performance, increased activation in the left inferior frontal gyrus as well as the left precuneus was found in risk-allele carriers in the semantic verbal fluency task.
Conclusions: The rs1006737 variant does influence language production on a semantic level in conjunction with the underlying neural systems. These findings are in line with results of studies in bipolar disorder, schizophrenia and major depression and may explain some of the cognitive and brain activation variation found in these disorders.

Genome-wide association study implicates chromosome 6p12.1 as a susceptibility locus for sarcoidosis in German population

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Sarcoidosis is a complex systemic inflammatory disease with a strong genetic component. To identify sarcoidosis susceptibility loci, we genotyped 116,204 SNPs in 381 sarcoidosis patients and 392 control individuals of German ancestry. We followed up the 25 most strongly associated SNPs in 1,582 sarcoidosis cases and 1,783 controls. Our results provide strong support for the association of one locus on chromosome 6p12.1 and sarcoidosis (nominal P value = 2.64x10-4 in the GWAS, and P = 1.17x10-3 in the validation panel). Extensive fine mapping of the novel locus and expression studies of the corresponding genes points to yet unidentified variants in a potential candidate gen as the most likely risk factor. Genetic and functional association of this gene with several kinds of cancer has been shown. Moreover, the corresponding protein is a key member of a pathway that has been implicated to play a role in chronic lung fibrosis and immune system communication. Our results extend the list of genetic loci implicated in sarcoidosis susceptibility and suggest a further promising target for study in related complex immune disorders.

Identification of novel candidate genes and molecular pathways in the pathogenesis of cardiomyopathy

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Cardiomyopathy with subsequent heart failure has become the leading cause for cardiac transplantation in the Western world. Using different genetic screening strategies it was possible to identify several cardiomyopathy-associated mutations. Most of these mutations affect proteins specifically expressed in striated muscle tissue.
In our approach we thus extracted the information about gene expression stored in public databases to identify novel cardiac- and muscle enriched genes. After exclusion of already characterised genes we defined a subset of 30 novel candidate genes out of a total of more than 400. For further analysis, tissue specific expression was validated by qRT-PCR. In the next step we focused on potential interacting partners using the yeast-two-hybrid technology. This allowed us to gain more information about possible function, interacting partners and cellular pathways. For subsequent in-depth analysis, we focused on those genes which revealed an interaction with already known cardiomyopathy disease genes. For these genes, subcellular localization and adenoviral overexpression / knockdown experiments were performed in rat cardiomyocytes. The most promising genes were chosen for in vivo experiments in zebrafish. As a result of our "pipeline", we were able to identify 3 new candidate genes which also displayed sarcomeric and /or cytoskeletal localization and showed a heart or muscle specific phenotype in the zebrafish model system. In one of these candidate genes we could identify several mutations in a collective a human patients samples with cardiomyopathy.
Taken together, this candidate gene approach may thus not only lead to the identification of novel disease genes for human cardiomyopathy, but should also allow to gain a deeper insight into the molecular network in the pathogenesis of cardiomyopathy.

Myomasp, a novel heart and muscle specific component of the M-Band involved in stretch sensing

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The M-band represents a transverse structure in the center of the sarcomeric A-band and provides cross-stabilisation of Actin- and Myosin-filaments. Although studies on the two M-band specific proteins Myomesin (MYOM1) and M-protein (MYOM2) revealed information about the structure of the M-band, its exact molecular composition and functional characteristics are still unclear. The M-band has also been implicated in mechanical stress sensing and signaling via SRF-dependent pathways.
Using a bioinformatic approach we identified a novel leucine rich protein, Myomasp (Myosin-interacting, M-band-associated stress-responsive protein) which is highly conserved between the species. Expression of Myomasp is specific for heart and muscle tissue as determined by qRT-PCR, northern- and western blot analyses. Immunolocalization of Myomasp revealed strong expression at the sarcomeric M-band. Yeast two-hybrid screening and co-immunoprecipitation identified the c-terminus of myosin heavy chain (MYH7) as an interaction partner.
This lead us to the hypothesis that Myomasp might not only be involved in the crosslinking of MYH7 to the M-band but may also modulate stretch sensing at the sarcomeric M-band. Knockdown of Myomasp in neonatal rat ventricular cardiomyocytes (NRVCMs) markedly downregulates mRNA expression of MYOM1 and MYOM2 and upregulates the stretch-sensitive markers GDF-15 and BNP as measured by qRT-PCR. Stimulation with hypertrophic agonists such as phenylephrine resulted in downregulation of Myomasp, MYOM1 and MYOM2 mRNA expression. Finally, the knockdown of Myomasp in NRVCM led to a dose dependent suppression of SRF-dependent gene expression.
In conclusion, we identified a novel heart and muscle specific M-band protein termed Myomasp, which interacts with MYH7 and negatively regulates stretch-sensitive genes like GDF-15 and BNP.

Hifi, a novel heart and muscle specific protein z-disk protein with anti-hypertrophic properties

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Cardiomyopathy with subsequent heart failure has become the leading cause for cardiac transplantation in the Western world. In order to unravel novel candidate genes for cardiomyopathy, we devised an “in silico” screen for cardiac and muscle enriched proteins and identified Hifi (hypertrophy inhibiting Filamin interactor) as a candidate. The predicted tissue specificity was further confirmed by qRT-PCR as well as northern- and western-blot analysis.
Hifi encodes for a 42kd protein which is highly conserved among species and localizes to the sarcomeric z-disc. Yeast two-hybrid screening and co-immunoprecipitation identified ?-filamin as an interacting partner.
To gain more insight into potential disease relevance we analysed the gene expression in mouse models for cardiomyopathies. For dilatative cardiomyopathy (DCM) we analysed Hifi mRNA expression in CSRP-KO mice; as model for hypertrophic cardiomyopathy (HCM) with progression into heat failure we used a constitutive active calcineurin transgenic mouse. In both cases we could detect a significant upregulation of HIFI compared to wildtype strains. For subsequent in-depth analysis of Hifi effects on HCM we analysed the mRNA expression of hypertrophic biomarkers in neonatal rat ventricular cardiomyocytes (NRVCM) after drug induced hypertrophy utilizing phenylephrin (PE) or angiotensin (ANG) stimulation and simultaneous adenoviral HIFI overexpression.
By the use of this in vitro approach we could detect a significant reduction of the hypertrophic response measured by qRT-PCR of the biomarkers ANF; BNP and MCIP. These results are further confirmed by the inhibition of ANF and NFAT- promoter activity in PE stimulated NRVCMs in the presence of HIFI in a dose dependent manner.
Taken together we identified a novel heart and muscle specific Z-disc protein which interacts with ?-filamin and is transcriptionally upregulated in disease models for cardiomyopaties and compensates for hypertrophic stimuli in vitro.

Functional analysis of IL31 promoter variants associated with eczema

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Interleukin-31 (IL-31) is a highly pruritogenic cytokine that has emerged as an important mediator in the pathogenesis of eczema. We previously identified association of an IL31 haplotype with nonatopic eczema. The haplotype tagging SNP, IL31-1066, is located in the promoter region within a consensus site for members of the GATA transcription factor family. To analyse the effect of the promoter haplotype on IL31 expression, we performed promoter studies using the luciferase reporter gene assay. Each of the promoter regions (2.3kb) of the three most common IL31 haplotypes (A=45,5% / B=29,4% / C=18,5%) was cloned into a luciferase reporter vector (pGL3) and transfected into the human Hodgkin lymphoma cell line L428. To determine the peak of expression of the luciferase gene, short-term kinetic analysis was performed at baseline and after stimulation (3/6/9/12/15/18h) with PMA. The highest expression was detected 15h after stimulation, which was used as the point of time measuring the luciferase activity in the following experiment. Luciferase activities of the three promoter constructs at baseline and after stimulation were compared over several experiments. No significant difference of the risk haplotype compared to the non-risk haplotypes was observed. Next, we performed the same set of experiments with the human T cell line Jurkat in order to exclude the probability of false positives emersed to the use of L428 cells. No differences in expression were found, even after specific stimulation with anti-CD3/anti-CD28. A potential effect caused by the 2,3kb promoter region could not be detected using the luciferase reporter gene assay in Jurkat and L428 cells. The genetic factor affecting IL-31 expression in nonatopic eczema might be located outside the cloned region or the cell lines used for the analysis might not be an appropriate biological system to detect altered expression of the IL31 haplotype.

Screening for functional polymorphisms in the epidermal differentiation complex identifies SPRR3 as a novel candidate gene for eczema

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Our genome-wide association study on eczema has recently suggested the existence of risk loci within the epidermal differentiation complex (EDC) on 1q21 apart from the well-established mutations in the filaggrin gene (FLG). In order to identify eczema-associated genes we searched the NCBI SNP database for polymorphisms within the EDC-genes, which were supposed to alter protein function significantly. We identified 13 frameshift mutations, 3 nonsense mutations, 2 stop codon mutations, and 1 larger insertion, which affected a total of 14 EDC-genes located between S100A10 and S100A1. Subsequently, we aimed to verify the polymorphisms in a set of 95 individuals with eczema. Only 3 polymorphisms could be validated, a nonsense mutation in FLG2, a 24bp-insertion in SPRR3, and a frameshift mutation in S100A3. The minor allele frequencies were 13.3%, 47,2%, and 0.8%, respectively. We then performed an association study by genotyping the three polymorphisms in 555 eczema cases and 375 unselected controls. We found a significant association with eczema for SPRR3 (p=0.02) but not for FLG2 and S100A3. Small proline rich proteins (SPRRs) are cross-bridging proteins in the cornified envelope which provides the main barrier function in stratified squamous epithelia. Individual members of the SPRR family are thought to specifically modulate the flexibility of this protective structure in different epithelia. Further studies are required to confirm the role of SPRR3 in eczema.

Myozap, a novel component of the intercalated disc, activates SRF-dependent signaling

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The intercalated disc (ID), the highly specialized cell-cell contact structure ensures mechanical and electrical coupling of rhythmically contracting cardiomyocytes. Recently, the ID has been recognized to be a hot spot of inherited cardiac disease, in particular arrhythmogenic right ventricular cardiomyopathy (ARVC) involving mutations in desmoplakin, plakophilin-2, desmoglein-2, desmocollin, and plakoglobin. We hypothesized that, given its complex structure and function, important constituents of the ID still remain unknown.

Using a bioinformatic screen, we discovered and cloned a novel 463 amino acid/54.2 kDa cardiac protein which we termed myozap (Myocardium-enriched ZO-1-associated protein). Myozap is strongly synthesized in the heart where it localizes to the ID which was shown by colocalizations with plakophilin-2, Plakoglobin and N-cadherin. Furthermore, it binds directly to and colocalizes with desmoplakin and ZO-1, two proteins of the ID that are implicated in the pathogenesis of human heart disease, including ARVC and heart failure. In a yeast-two hybrid screen for additional binding partners of myozap we identified myosin phosphatase-RhoA interacting protein (MRIP), a negative regulator of Rho activity. Myozap, in turn, strongly activates SRF-dependent transcription through its ERM (Ezrin/radixin/moesin)-like domain in a Rho-dependent fashion. Conversely, the addition of MRIP led to a significant decrease of the activation of the SRF-dependent sm22-reporter gene.

These findings reveal myozap as a previously unrecognized component of a Rho-dependent signaling pathway that links the intercalated disc to cardiac gene regulation. Given its subcellular location and myriad binding partners involved in the function of the ID, myozap represents a likely mediator of cardiomyopathies. This notion is further supported by a cardiomyopathic phenotype with progressive loss of contractile function induced by Myozap orthologue knockdown in zebrafish.

Deamination and methlyation of CpGs drive formation of Myc and p53-binding sites.

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Tumor suppressor genes such as p53 contain methylated CpG dinucleotides and these define mutational hot spots in human cancers. Here, we show that methylation and deamination of CpGs generate in vivo p53 and Myc -binding sites on a genomic scale. Since promoter hypermethylation is the best characterized epigenetic change to occur in tumors, we propose that the deamination of methylated CpGs could result in de novo generation of the transcription-factor-binding sites and contribute to gene deregulation.

Assessment of Alzheimer's disease GWAS findings in a large collection of family-based samples

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Within the past two years, a total of 11 independent genome-wide association studies have been published in the field of Alzheimer's disease (AD), highlighting over two dozen novel potential susceptibility loci beyond the well-established APOE association. Currently, the most compelling GWAS signals have been observed in CLU (APOJ), PICALM, CR1, and GAB2 (for an up-to-date overview of these an other genetic association findings in AD please consult the AlzGene database maintain by our group at www.alzgene.org). However, the vast majority of these signals emerged from heterogenous multicenter case-control studies, which lack independent replication in samples ascertained from multiplex families. Family-based methods have the advantage of being robust against bias due to undetected population stratification and phenotype misspecifications. The purpose of this study was to assess these all of the most compelling AD GWAS signals in over 4,000 DNAs from nearly 1,300 independent multiplex AD families or discordant sibships. Overall, we directly genotyped over 40 SNPs across 25 different GWAS loci in these samples using a combination of single (high-efficiency fluorescence polarization) and multiplex (OpenArray) assays. Association testing (using PBAT) revealed significant results confirming the previous associations between risk for AD and variants in CLU, CR1, and GAB2, in addition to several other loci. Notably, these associations were found with the same alleles and the same direction of effect as in the original reports. The independent convergence of case-control GWAS and family-based follow-up findings sets these loci apart from any of the other >20 currently discussed AD GWAS findings, and substantially strengthens the notion that these loci exert genuine effects on AD pathogenesis.

MSGene – A novel and publicly available database of genetic association studies in multiple sclerosis

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Multiple sclerosis (MS) is a genetically complex disorder with both genetic and environmental factors contributing to disease susceptibility. In the past two decades, literally hundreds of reports have been published claiming or refuting genetic association between putative MS genes and disease risk, or other phenotypic variables. In addition, several genome-wide association studies (GWAS) have been reported, highlighting putative novel MS loci that need further validation. This wealth of information is becoming increasingly difficult to follow, evaluate, and most importantly to interpret.

To this end, based on methodology previously developed by our group, we are currently building a database (MSGene), which will serve as a regularly updated resource of MS genetic association studies. Data for MSGene will be gathered following systematic searches of the scientific literature, and will summarize essential characteristics of each study as well as gene-specific results including genotype frequencies. Following this strategy, we have now systematically collected over 800 MS genetic association papers reporting on over 2000 polymorphisms across 505 loci. In addition to these candidate gene-based studies, MSGene also provides detailed summaries of the five currently published GWAS. An integral part of MSGene is the systematic computation of random-effects meta-analyses for all polymorphisms with sufficient genotype data. Of the 93 meta-analyses currently available in MSGene, 17 genetic variants in 14 loci excluding HLA show significant effects with odds ratios ranging from 1.1 to 1.6; nearly half of these were originally implicated by GWAS (e.g. CLEC16A, IL7R). Data collected as part of this project - including the results of the up-to-date meta-analyses - will be publicly available at http://www.msgene.org at the time of this meeting. We will provide a synopsis of the current status of the MS genetics field, with a focus on loci showing significant summary odds ratios.

Systematic integration of GWAS data into meta-analyses of Parkinson's disease genetic association studies

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Our group has created and maintains a continuously updated online database for genetic association studies in Parkinson's disease (PDGene; available at www.pdgene.org). One of the goals of PDGene is to provide unbiased and up-to-date meta-analyses for polymorphisms with sufficient published genotype data. A particular challenge in this context is the systematic integration of genome-wide association studies (GWAS) into the existing, mostly candidate-gene based meta-analyses. On September 1st, 2009, PDGene included data from nearly 800 publications reporting on over 2,000 different polymorphisms of which 144 across 63 genetic loci had sufficient data to warrant meta-analysis. Thirty-four of the meta-analyses showed significant risk effects with summary odds ratios (ORs) ranging from ~1.1 to 3.1. Adding publicly available GWAS data (i.e. a total of 601,000 non-redundant SNPs) from three studies (Maraganore, 2005; Fung, 2006; Pankratz, 2008) overlapping with polymorphisms already included in PDGene, increased the number of meta-analyes by nearly 40% to 201. Of these, 40 across 23 loci showed evidence for significant association with PD risk. Notably, some meta-analyses significant before inclusion of the GWAS data were no longer supported after the merge (e.g. MTHFR and PHACTR2), while others newly emerged as significant (e.g. ABCB1, BDNF, GLIS1 and PDXK). To assess the epidemiologic credibility of all 40 positive results, we applied grading criteria recently developed by the Human Genome Epidemiology Network. In addition to several established PD risk genes (e.g. SNCA, MAPT, and LRRK2), the highest credibility grading was also assigned to the newly meta-analyzed polymorphism in BDNF. These results show that integrating GWAS data into systematic meta-analyses of candidate gene studies can substantially augment the power of gene-finding efforts in complex diseases.

Disruption of ST5 is associated with mental-retardation and multiple congenital anomalies

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We observed a patient with a cryptic subtelomeric de novo balanced translocation 46,XY.ish t(11;20)(p15.4;q13.2) presenting with severe mental retardation, muscular hypotonia, seizures, bilateral sensorineural hearing loss, submucous cleft palate, persistent ductus Botalli, unilateral cystic kidney dysplasia and frequent infections. Fluorescence in situ hybridization mapping and sequencing of the translocation breakpoints showed that no known genes are disrupted at 20q13.2 and that ST5 (suppression of tumorigenicity 5; MIM 140750) is disrupted on 11p15.4. By quantitative PCR from different human tissues we found ST5 relatively evenly expressed in fetal tissues. ST5 expression was more pronounced in adult brain, kidney and muscle than in the corresponding fetal tissues, whereas expression in other tissues was generally lower than in the fetal tissue. Using RNA in situ hybridization in mouse we found that St5 is expressed in the frontal cortex during embryonic development. In adult mouse brain expression of St5 was especially high in the hippocampal area and cerebellum. Hence we suppose that ST5 plays an important role in central nervous system development probably due to disturbance of DENN-domain mediated vesicle formation and neurotransmitter trafficking. Thus, our findings implicate ST5 in the etiology of mental retardation, seizures and multiple congenital anomalies.

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Poster presentation abstracts

Symposium II

Genomics of Sporadic cancer

Genome-Wide Profiling of Astrocytic Gliomas Reveals Frequent RPRM Silencing in TP53 Wild-Type Tumors and Molecular Signatures Associated with Tumor Entity and IDH1 Mutation Status

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PURPOSE: To identify novel pathomechanisms and molecular markers in astrocytic gliomas on the basis of genome-wide profiling approaches.
PATIENTS AND METHODS: Genomic aberrations were determined by array-based comparative genomic hybridization in 131 diffuse astrocytic gliomas, including 87 primary glioblastomas (pGBIV), 13 secondary glioblastomas (sGBIV), 19 anaplastic astrocytomas (AAIII), and 12 diffuse astrocytomas (AII). All tumors were screened for IDH1 and IDH2 mutations. Expression profiling was performed for 74 tumors (42 pGBIV, 11 sGBIV, 13 AAIII, 8 AII). Unsupervised and supervised bioinformatic analyses were used to identify genomic and expression patterns linked to tumor entity, IDH-gene mutation status, and overall survival (OS). Two newly identified candidate genes (RPRM, FGFR2) were subjected to focused molecular analyses.
RESULTS: Distinct genomic and expression profiles separated pGBIV from the other entities. Classifier expression signatures were closely associated with IDH1 gene mutation status. The subtype of IDH1-mutant pGBIV shared expression profiles with sGBIV and had longer OS than IDH1 wild-type pGBIV. The RPRM gene encoding the p53-induced protein reprimo was frequently hypermethylated and transcriptionally down-regulated in TP53 wild-type gliomas, including most pGBIV. Furthermore, FGFR2 was homozygously deleted in two pGBIV, with reduced FGFR2 mRNA levels being frequent in pGBIV and associated with poor outcome.

DUSP4/MKP-2 is inactivated by DNA hypermethylation and acts as a growth suppressor in glioma cells

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In a genome-wide screen using differential methylation hybridization (DMH) we identified a CpG-rich fragment within the promoter region of the dual-specificity phosphatase 4/map kinase phosphatase 2 gene (DUSP4/MKP-2) that showed hypermethylation in gliomas. Bisulfite sequencing and COBRA (combined bisulfite restriction analysis) were performed to confirm the methylation status in 83 astrocytic gliomas of different malignancy grades and five glioma cell lines. Hypermethylation of DUSP4/MKP-2 was significantly more frequent in diffuse and anaplastic astrocytomas as well as secondary glioblastomas as compared to primary glioblastomas ($p < 0.001$) and associated with prolonged survival in the group of primary glioblastoma patients. DUSP4/MKP-2 methylation was associated with TP53 and IDH1 mutation and exclusive of EGFR amplification. Investigation of DUSP4/MKP-2 expression revealed a significant association between hypermethylation and reduced mRNA and protein levels in gliomas. Treatment of DUSP4/MKP-2 hypermethylated glioma cell lines with 5-aza-2'-deoxycytidine increased DUSP4/MKP-2 mRNA expression. Furthermore, overexpression of DUSP4/MKP-2 inhibited proliferation in the glioblastoma cell lines U373MG and A172. In summary we have identified DUSP4/MKP-2 as a novel epigenetically silenced gene in gliomas. Its frequent inactivation as well as its inhibitory effect on tumor cell proliferation and focus formation strongly argues for a significant role in glioma development.

EMP3 – A putative Tumor Suppressor?

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The epithelial membrane protein 3 (EMP3) was identified as potential tumor suppressor in gliomas. The gene is located on chromosome 19q, in a region which is known to undergo heterozygous deletion (LOH) in a subset of gliomas. The EMP3 promoter is often methylated in these gliomas thereby silencing expression. These findings support the hypothesis that EMP3 functions as a tumor suppressor. However nothing is known about the physiological function of EMP3.
The aim of our work is the functional characterization of EMP3. First experimental data revealed quite opposite expression levels of EMP3 in different tumor models. For example high EMP3 RNA levels are observed in mammospheres while that in neurospheres is low. Interestingly, differentiation of neurospheres towards glial lineage cells is accompanied by a strong elevation of EMP3 expression. Thus EMP3 might be involved in such differentiation. We detected an interesting relation between EMP3 expression and the mutational status of IDH1. IDH1 mutations are the most frequent genetic alteration in oligodendroglial and diffuse astrocytic tumors of WHO grades II and III and are believed to constitute very early steps in tumorigenesis occurring in precursor cells capable of both, later progression towards oligodendroglial and astrocytic tumors. Gliomas with IDH1 mutation have strong EMP3 promoter methylation not seen in these tumors without this mutation ($p < 0.0001$). Again this may indicate that EMP3 contributes to differentiation.

Function and regulation of PRDX1 in chemo- and radiosensitivity of glial tumours

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Oligodendrogliomas (OD) are primary glial brain tumours that are divided into well-differentiated (WHO grade II) and anaplastic (WHO grade III) tumours, which very frequently demonstrate allelic losses (LOH) on chromosomal arms 1p and 19q. These deletions are strongly associated with favourable response to radio- and chemotherapy as well as prolonged survival.
To resolve the underlying molecular mechanisms, we investigated expression profiles of OD with LOH1p19q in comparison to OD without these deletions. These experiments revealed that the peroxiredoxin 1 (PRDX1) gene located on 1p34.1 showed the most significant down-regulation in OD with LOH1p19q. The antioxidative function of PRDX1, which scavenges hydrogen peroxide, is discussed to be involved in the pathogenesis and therapeutic response of different tumour entities. The generation of reactive oxygen species (ROS) contributes to different treatment strategies as high ROS levels can lead to severe cell damage and apoptosis. Therefore, abnormal regulation of antioxidative enzymes can contribute to resistance and thus influences therapeutic outcome. To gain further insights into the regulation of PRDX1 in OD we analysed the PRDX1 promoter methylation state of patients with WHO grade II and III gliomas by sodium bisulfite sequencing. Thereby, we found a strong association of loss of one allele of PRDX1 and epigenetic silencing of the remaining copy, which points to a crucial role of PRDX1 inactivation in OD. To assess the function of PRDX1 in OD, we established a stable knockdown of PRDX1 in glioma cell lines. Our data clearly demonstrate a significant increase in apoptosis upon application of chemotherapeutic agents and gamma-irradiation after PRDX1 knockdown in vitro. Consequently, we postulate that OD with low PRDX1 expression are deficient in ROS-detoxification during radio- and chemotherapy, which results in increased cell death and consecutively in a better therapeutic response.

Integration of miRNAs into the ERBB receptor network for combinatorial targeted therapy

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Aberrant ERBB receptor signaling can induce development of many human cancers e.g. breast, lung, brain and gastric carcinomas. Particularly, EGFR and ERBB2 belong to the most oncogenic kinases and are effective targets for the treatment of cancer, but the response rate to these targeted therapies is rather low. Recently, a novel class of gene regulators was discovered and named as microRNAs (miRNAs), which are ~22 nucleotides in length and regulate the expression of genes post-transcriptionally. Up to now, several miRNAs (e.g. miR-7 and miR-205) have been shown to regulate components of the ERBB receptor network and related cellular processes. Here, we aim at identifying miRNAs which regulate the ERBB receptor network and presenting them as potential drug targets for effective combinatorial targeted therapy.

We started with constructing the ERBB receptor network including all ERBB receptor family members, downstream signaling intermediates, negative feedback regulators, key TFs and cell cycle proteins. Using miRNA mimic library containing 810 miRNAs, we screen the effects of each miRNA on the expression levels of network components using Reverse Phase Protein Arrays (RPPAs) and qRT-PCR in the EGFR-overexpressing MDA-MB-231 breast cancer cell line. Direct targeting of miRNAs is validated in luciferase reporter assay as well as site-directed mutagenesis, and identified miRNAs/targets will be studied in different cancer-relevant cell-based assays. Finally, we will test the effects of miRNAs/targets in combination with several ERBB receptor-targeting drugs e.g. gefitinib or trastuzumab in different cell line models (drug sensitive vs. resistant). Hence, combining bioinformatics, functional genomics, proteomics as well as molecular and cellular biology approaches, we integrate miRNAs into the well-studied ERBB receptor network for combinatorial targeted therapy.

Localisation- and mutation-dependent microRNA (miRNA) expression signatures in gastrointestinal stromal tumours (GISTs), with a cluster of coexpressed miRNAs located at 14q32.31

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The molecular biology and clinical behaviour of gastrointestinal stromal tumours (GISTs) are associated with their anatomical localisation (stomach or intestine), and also with the mutation status of the receptor tyrosine kinases KIT and PDGFRA. Twelve GISTs were evaluated for differential miRNA expression signatures by use of microarrays representing 734 human miRNAs. Thirty-two miRNAs were found to be differentially expressed according to localisation and mutation status. Differential expression was further analysed and confirmed for four miRNAs (miR-132, miR-221, miR-222, and miR 504) by qRT-PCR in 49 additional GISTs. Differentially expressed miRNAs were functionally mapped to KIT/PDGFRA signalling and G1/S-phase transition of the cell cycle, revealing 22 predicted miRNA/mRNA interactions for ten gene targets from KIT/PDGFRA signalling, and twelve interactions for twelve gene targets of G1/S-phase transition. Moreover, the expression of 44 miRNAs clustered in a genetically imprinted region at 14q32.31 was found to be strongly correlated in the microarray analysis. This was confirmed for two selected miRNAs (miR-134 and miR-370) from the 14q32.31 cluster by qRT-PCR in 49 additional GISTs, and the expression of these two miRNAs was significantly lower in GISTs with 14q loss, and also in GISTs with tumour progress.

Circulating miRNAs are correlated with tumor progression in prostate cancer

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Circulating miRNAs have recently been indicated as practicable and promising biomarkers for non-invasive diagnosis in various tumor entities. However, cell-free miRNAs have not been found to correlate with clinico-pathological variables in epithelial carcinomas. In order to learn more about the potential clinical relevance of circulating miRNAs in prostate cancer, we screened 667 miRNAs in serum samples from patients with metastatic (n=7) and localized prostate cancer (n=14). Various miRNAs were highly abundant in the sera of patients with metastatic disease. Five miRNAs were further analyzed in an independent patient cohort (n=45). One of these turned out to be the most pronounced serum marker associated with tumor progression, and its level correlated with Gleason scores and lymph-node status of the patients´ tumors. Analysis of variance (ANOVA) indicated a benefit for the prediction of different clinical parameters when the circulating miRNA was combined with prostate specific antigen (PSA) measurements. In addition, the expression level of this miRNA was found to be significantly higher in prostate tumor compared to normal tissue samples. Overall, our observations suggest that circulating miRNAs are associated with advanced prostate cancer disease.

Influence of estrogen metabolism, binding and signalling related single nucleotide polymorphisms on the risk of colorectal cancer

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Introduction: Colorectal cancer (CRC) is the third most common cancer type worldwide and its incidence varies among different populations and among genders. Exogenous hormone use has been associated with reduced CRC risk; therefore sex-steroids may play an important role in colon carcinogenesis [1]. We hypothesize that polymorphisms in estrogen metabolism and cellular binding genes influence the level of hormone exposure and therefore modify associated CRC risk. Thus we aim to explore effect modification of different hormone related factors by polymorphisms in selected genes on the risk of CRC in women.
Methods: 41 single nucleotide polymorphisms (SNPs) in the COMT, CYP17A1, CYP1A1, CYP1A2, CYP1B1, CYP2C19, CYP2C9, CYP3A4, ESR1, ESR2, GSTP1, HSD17B1, NIR1I2, PGR, SHBG genes were genotyped in study subjects from a German population-based case-control study (DACHS Study) using Kaspar assays (KBiosciences). Main effects of single SNPs were analysed in 734 female CRC patients and 721 healthy controls. Conditional logistic regression stratified for matching variables (5-year-age group, county of residence) was performed using both log-additive and co-dominant models to account for different modes of inheritance.
Results: In preliminary analyses, the genotypes of three SNPs showed a significant trend in risk for CRC in women ($p<0.05$). Carriers of rs743572 in CYP17A1 and carriers of rs605059 in HSD17B1 were at increased risk for CRC while risk for CRC was decreased in carriers of rs1255998 in ESR2.
Conclusion: Polymorphisms in genes of estrogen metabolism (CYP17A1, HSD17B1), binding and signalling (ESR2) may influence the risk for CRC in German women. At the NGFN+ meeting, we will be able to present the final, adjusted results.

References:
1. Kennelly, R., et al. Lancet Oncol, 2008; 9(4): 385-91

Genome-wide association study for colorectal cancer in German familial cases and replication of candidate markers in independent cohorts

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Rare high-penetrance germline mutations account for less than 5% of colorectal cancer (CRC) cases. Much of the remaining variation in genetic risk is supposed to be attributable to multiple common susceptibility loci. We performed a genome-wide association study (GWAS) with 371 German familial CRC cases and 1263 healthy controls using the Affymetrix 6.0 Array. SNPs with MAF <0.05, call rate <90%, or Hardy-Weinberg equilibrium p-value <10-5 in the control group, as well as samples with call rate <90%, were excluded.
No peaks could be identified in the Manhattan plot at genome-wide level (p-value 5x10-7). From the 523 SNPs with p-value <10-4 and reliable clustering plots, we selected markers for replication using the following criteria: several SNPs within/close to a gene, location in candidate genes or in already known risk loci for CRC, and coding polymorphisms. A total of 53 markers were genotyped in a German cohort of 575 familial CRC cases and 760 healthy controls using KASPar assays. At allele level, seven polymorphisms showed association (p-value 0.049 to 0.0001); two of them located at the known risk loci 8q24.21 and 11q23. From the other five, three were selected for further replications in a total of 4340 CRC cases and 4847 healthy controls. Taking all replications together (four cases and five different control groups), one of the three SNPs reached borderline significance (p allele 0.05; OR = 1.06 [1.00-1.12]).
Trying to increase the power to detect association between genes and disease, we applied the recently published approach ALIGATOR (Holmans et al., Am J Hum Genet 2009), which considers the biological pathway as a unit of analysis. The method successfully detected overrepresentation of MAPK signalling pathways, which are known to be implicated in CRC etiology, among the most significant SNPs (p allele <0.001) from our GWAS. SNPs belonging to involved genes are being further investigated.

Global Hypomethylation of the DNA is a Hallmark of Colorectal Carcinoma

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DNA methylation leads to a condensed chromatin structure preventing the interaction of DNA binding proteins with the DNA. Therefore, it contributes to e.g. genome stability or regulation of gene expression. Consequently, aberrations in the DNA methylation pattern are a typical hallmark of cancer including CRC [1, 2].
We have applied the luminometric methylation assay (LUMA) to analyze the global DNA methylation level in yet 42 cases of CRC and corresponding control tissues from the same patients.
LUMA is based on a digestion of the DNA with a combination of DNA methylation sensitive and insensitive restriction endonucleases. Subsequently, the relative number of cuts created by a methylation sensitive endonuclease and its insensitive isoschizomers can be analyzed by pyrosequencing to determine the global DNA methylation level [3, 4].
We found a significant global hypomethylation of the DNA in CRC compared to corresponding controls ($p<0.0001$). As expected, normal colon tissue showed a global methylation level between 70-80%. In contrast, the methylation level in the CRC samples was decreased down to 54-75%.
These aberrations in the DNA methylation pattern might contribute to both, alterations in the gene expression pattern and the genomic instability in CRC.

[1] Martin-Subero et al., 2009; PLoS ONE 4(9): e6986.
[2] Irizarry et al., 2009; Nat Genet. 41(2):178-86.
[3] Ammerpohl et al., 2009; Biochim Biophys Acta. 1790(9):847-62.
[4] Karimi et al., 2006; Exp Cell Res. 312(11):1989-95.

Dissecting Wnt Signalling in colon cancer cells by genome-wide RNAi and RNAseq

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Wnt signalling is a major factor in the development of cancer and especially in colorectal carcinoma (CRC). To elucidate the role of Wnt signalling in CRC we defined a transcriptional signature of Wnt signalling through RNAi of major pathway components. We combine RNAseq with RNAi of pathway components in cultured colon cancer cells. These profiles are then used to epistatically map and characterize novel pathway components.
A genome-wide RNAi screen colon cancer cells was performed to identify novel factors. These factors were further characterized by their effect on the defined transcriptional signature to confirm their phenotype. The Wnt signalling dependent gene expression profiles in colon cells can be used to elucidate the underlying function of Wnt signalling in CRCs.

Genetic and Epigenetic Alterations in Colon Cancer

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Changes in DNA methylation patterns of specific genes are commonly found during colorectal cancer progression. The recent development of next generation sequencing (NGS) technologies has brought about the possibility to investigate DNA methylation alterations in a genome-wide and unbiased manner. One of these technologies, the MeDIP technology, utilizes an antibody against methylated cytosine to immunoprecipitate and thereby enrich methylated DNA regions. We have adapted the MeDIP protocol to Illumina's Genome Analyzer as well as Applied Biosystem's SOLiD platform to identify precipitated DNA fragments in colon cancer cell lines as well as colon normal and tumor tissue samples. The combination of genetic and epigenetic experiments brings further access towards the identification of novel disease-causing genomic alterations in a genetically complex disease such as cancer.

Analyzing the regulation of metabolic pathways in neuroblastoma tumors

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Gene expression profiling by microarrays or transcript sequencing enables observing the pathogenic function of tumors on a mesoscopic level. We investigated neuroblastoma tumors which clinically exhibit a very heterogeneous course ranging from rapid growth with fatal outcome to spontaneous regression and detected regulatory oncogenetic shifts in their metabolic networks. In contrast to common enrichment tests, we took network topology into account by applying adjusted wavelet transforms on an elaborated and new two-dimensional grid representation of curated pathway maps from the Kyoto Enzyclopedia of Genes and Genomes. The aggressive form of the tumors showed regulatory shifts for purine and pyrimidine biosynthesis as well as folate-mediated metabolism of the one-carbon pool in respect to increased nucleotide production. We spotted an oncogentic regulatory switch in glutamate metabolism for which we provided experimental validation, being the first steps towards a new possible drug therapy. The pattern recognition method we used is superior to classical enrichment tests as it offers a much higher sensitivity for detecting such functionally related regulation patterns. Additionally, it is more precise to a permutation based enrichment method. The software is publically available as the R-package PathWave at www.ichip.de.

Accurate Prediction of Neuroblastoma Outcome based on miRNA Expression Profiles

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For neuroblastoma, the most common extracranial tumour of childhood, identification of new biomarkers and potential therapeutic targets is mandatory to improve risk stratification and survival rates. MicroRNAs are deregulated in most cancers, including neuroblastoma. We here analyse 430 miRNAs in 68 neuroblastomas by multiplex real-time PCR. Prediction of event-free survival (EFS) with support vector machines (SVM) and actual survival times with Cox regression-based models (CASPAR) were highly accurate and independently validated. SVM-accuracy was 86.4%(95%CI:85.7-87.1) and 5y-EFS probability in the CASPAR-predicted short survival group was 0.185(95%CI:0.0-0.383) compared to 0.782(95%CI:0.635-0.929) in the CASPAR-predicted long survival group. Amplification of the MYCN oncogene was highly correlated with deregulation of miRNA expression. In addition, 37 miRNAs correlated with TrkA expression, a marker of excellent outcome, and 6 miRNAs further analysed in vitro were regulated upon TrkA transfection, suggesting a functional relationship. Among them was miR-542-5p, a miRNA inversely correlated with MYCN amplification and event-free survival. We conclude that neuroblastoma patient outcome prediction using miRNA expression is feasible and effective. Studies testing miRNA-based predictors in comparison to and in combination with mRNA and aCGH information should be initiated. Specific miRNAs (e.g. miR-542-5p) might be important in neuroblastoma tumour biology, and qualify as potential therapeutic targets.

Histone deacetylase 8 in neuroblastoma tumorigenesis

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The effects of pan-histone deacetylase (HDAC) inhibitors on cancer cells have shown that HDACs are involved in fundamental tumor biological processes such as cell cycle control, differentiation and apoptosis. However, due to the unselective nature of these compounds, little is known about the contribution of individual HDAC family members to tumorigenesis and progression. The purpose of this study was to evaluate the role of individual HDACs in neuroblastoma tumorigenesis. We have investigated the mRNA expression of all HDAC1-11 family members in a large cohort of primary neuroblastoma samples covering the full spectrum of the disease. HDACs associated with disease stage and survival, were subsequently functionally evaluated in cell culture models. Only HDAC8 expression was significantly correlated with advanced disease and metastasis, and downregulated in stage 4S neuroblastoma associated with spontaneous regression. High HDAC8 expression was associated with poor prognostic markers, poor overall and event-free survival. Knockdown of HDAC8 resulted in inhibition of proliferation, in reduced clonogenic growth, cell cycle arrest and differentiation in cultured neuroblastoma cells. Treatment of neuroblastoma cell lines as well as short term culture neuroblastoma cells with a HDAC8 selective small molecule inhibitor inhibited cell proliferation, clone formation and induced differentiation, and thus reproduced the HDAC8 knockdown phenotype. Global histone 4 acetylation was not affected by HDAC8 knockdown or by selective inhibitor treatment. Our data point toward an important role of HDAC8 in neuroblastoma pathogenesis and identifies this HDAC family member as a specific drug target for differentiation therapy of neuroblastoma.

Oehme, I., et al. Histone deacetylase 8 in neuroblastoma tumorigenesis. Clin Cancer Res, 15: 91-99, 2009.

Genome-wide RNAi Screen Identifies a New Component of the Wnt/ß-Catenin Signaling Pathway

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Wnt (wingless-related mouse mammary tumor virus integration site) proteins form a family of highly conserved secreted glycoproteins involved in embryonic development, stem cell regulation and carcinogenesis. Wnt proteins bind to cell surface receptors and trigger activation of canonical Wnt signaling through ß-catenin. Members of the Wnt pathway have shown to be mutated and deregulated in cancer. Although its significance in development and tumorigenesis is underlined with the growing number of reported mutations in different pathway components, mechanisms and regulators of Wnt secretion, reception, signal relay and activator complex formation remain not fully understood.

RNA interference (RNAi) has become a powerful tool to silence gene expression. Genome-wide RNAi screening, in combination with cellular assays, bridges the gap between systems biology and traditional single-gene-based functional analysis. We make use of RNAi as a screening approach to identify new regulators of the Wnt signaling pathway.

We present HEZ1 as a newly described putative Wnt cascade component. Our results show that HEZ1 modulates the stability of ß-catenin; loss of HEZ1 function decreases active ß-catenin levels in the cell. Future steps include functional characterization of HEZ1 in vitro and genetic analysis in Drosophila melanogaster of its fly homologue. Elucidation of the role of HEZ1 may contribute to our understanding of ß-catenin regulation in the context of Wnt signaling.

Functional characterisation of cancer-relevant mutations using recombinant cancer cell line technology

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Cancer is the major cause of death in the industrialised world and somatic mutations play key roles in the development and the progression of this disease. In recent years, a multitude of mutational profiling studies has been performed in order to elucidate clinically relevant genes and their most frequent mutations. However, the detailed functional consequences of most of these genetic alterations on cellular processes remained unknown.

In order to analyse molecular changes associated with potentially cancer-relevant mutations, we developed a powerful recombination-based system to generate libraries of stably transfected cell lines. By inserting genes of interest into a pre-defined locus within the cellular genome, we are able to generate highly standardized series of isogenic cell lines. Within the NGFNplus project IG-Mutanom we use this technology and so far constructed a breast cancer cell line library consisting of 50 recombinants inducibly overexpressing different mutated cancer genes and their wild type counterparts. In an initial screen, this set of candidate genes is analysed for effects on the viability of the tumour cells. By combining the results of this functional assay with the outcome of analyses on the protein- and mRNA-level, we aim at deepening our understanding on the complex networks of signalling pathways associated with cancer development and progression.

Hsp90 function inhibition reveals differential increased protein stability in cancer related signaling pathways of lung, colon and osteosarcoma cancer cells

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The heat shock protein 90 (Hsp90) plays an important role for the cellular signaling of kinases and hormone receptors. In cancer cells mutated and inherently instable oncogenes are stabilized by Hsp90 and are in most cases degraded after Hsp90 inhibition, making Hsp90 an attractive target of cancer therapy. We assessed the consequences of Hsp90 inhibition on (a) the proteome level of 1187 proteins using isobaric tags (iTRAQ) quantitative mass spectrometry and (b) on 76 kinases by quantitative Western blotting. From this work we present eight novel potential client proteins. On a proteome level Hsp90 inhibition affects cellular signaling by altering the protein levels of major key regulatory molecules in DNA repair, transcriptional regulation and protein translation. To get a more analytical insight into the role of Hsp90 for tumorgenesis-relevant pathways we assayed the differences between primary and cancer cells comparing human primary fibroblasts (Hs68) with three cancer cell lines (SW480, A549, U2OS). The cancer cell lines show a much-reduced susceptibility to Hsp90 inhibition in 6 different growth and cancer related pathways as compared to the primary cells. This supports the idea that cancer cells have accumulated Hsp90-related traits that make them more self-sufficient. Our work shows that future diagnostic and chemotherapeutic approaches will need to take into account cancer specific modulations of Hsp90 function that influence cellular signaling and growth.

Proteomic and functional characterization of oncogenes and tumor suppressors in a cancer tissue culture model in the IG Mutanom project

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The pathogenesis of cancer is coupled to the accumulation of mutations over the lifetime of an organism. International sequencing efforts identified a large number of cancer-associated mutations in a wide variety of tumor types. In spite of these efforts, the functional relevance and molecular consequences of these mutations often remain to be investigated. The Mutanom consortium (www.mutanom.org) aims to characterize frequently occurring mutations in a systems biology approach. This project combines genomics, proteomics, cellular assays with data from model organisms and the clinic.
We use isogenic tissue culture acceptor cells (e.g. Flp-In T-Rex) to generate stable cell lines expressing normal or mutated oncogenes/tumor suppressors under the control of a tetracycline inducible promoter. This method produces cell lines at high efficiency for controlled experiments within the same genetic background, while highly comparable conditions are maintained for biochemical and functional characterization of the target genes/proteins.
With this at hand, we identify differences in protein complex composition using Tandem Affinity Purification (TAP) followed by mass spectrometry. The use of isobaric labeling allows the quantitative analysis of complex composition, comparing wild type and mutant protein. In addition, proteome profiling and KinobeadsTM approaches provide a quantitative readout on the effect of oncogene/tumor suppressor expression for a large cohort of proteins and enriched kinases, respectively. The main pathways we are currently analyzing are the PI3-kinase pathway and the DNA damage response, which are responsible for cell growth and survival as well as surveillance of genome integrity, respectively. Results are integrated in a systems biology approach with data obtained from proteome analyses, second generation sequencing and mRNA profiling experiments to generate a predictive model of cancer pathways.

Next Generation Sequencing of Coding Regions in a Colon Cancer Patient

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According to the world health organization (WHO) colon cancer is the third most common cancer worldwide with an incidence of 1.080.000 cases in 2004. Undoubtedly genetic and epigenetic factors play an important role in the origin and/or progression of colon cancer. To this end, we have performed whole exome sequencing of a colon cancer patient using microarray-based sequence capturing of ~180,000 coding regions followed by massively parallel sequencing using the Roche/454 FLX Genome Sequencer. We sequenced normal and tumor colon tissue from the same patient and developed analyses pipelines especially adapted to tumor tissues. Our sequencing output with 2 Gb and a median read length of 415 bases per 454 run corresponds to high technical standards. We identified approximately 70,000 single nucleotide polymorphisms, 9-13% of which are new and not listed in dbSNP. Overall approximately 10% represent non-synonymous substitutions within the coding sequence, whereas approximately 70% are located in adjacent non-coding sequences or micro-RNA regions. In addition, we accurately identified ~5600 small-scale (1-49bp) insertion and deletion polymorphisms. Using pathway analysis tools we see a primary accumulation of genomic alterations in tumor relevant signalling pathways suggesting a multi-hit strategy for tumor development. This project is embedded in the IG Systems Biology of Genetic Diseases – Mutanom. Within this project identified mutations will be functionally characterized and their effect on protein-protein interactions will be studied using tandem affinity purification (TAP) and yeast two hybrid methods.

Strand-specific RNA sequencing of HepG2 cells identifies genes that are differentially expressed, alternatively spliced and allelically imbalanced in response to TGF-beta

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TGF-beta is a secreted protein that controls many complex behaviors of tumor cells. HepG2, a human hepatocellular carcinoma cell line, has lost responsiveness to the tumor suppressive action of TGF-beta and when treated with TGF-beta, these cells show enhanced motility and invasiveness. Thus, HepG2 cells provide a good model for advanced tumor cells. RNA sequencing (RNA-Seq) can provide a detailed view of the dynamic transcriptional landscape of cells experiencing environmental perturbation and was therefore applied here to study TGF-beta response. We serum starved HepG2 cells for 24 hours and then treated for 1 hour with 2.5 ng/ml TGF-beta1 or with vehicle control. Total RNA was isolated, depleted of rRNA, fragmented and then used to create strand-specific cDNA libraries with the SOLiD ™ Whole Transcriptome Protocol. Approximately 500 million 50 bp reads were sequenced with the SOLiD ™ System, and showed alignment to the human genome. The expression level of each gene was defined and 349 genes were determined to be differentially expressed between the two conditions. The 50 bp length of the sequenced fragments permitted us to accurately align reads to splice junctions. In all, ~5 million reads aligned to ~90,000 splice junctions in both the control and stimulated conditions, which allowed us to determine the effects of TGF-beta on alternative splicing in fine detail. Finally, by genotyping HepG2 cells, we have identified over 300,000 heterozygous SNP positions, which we have employed to examine allele-specific gene expression in our RNA-Seq data. We observed many differences in the balance of expressed alleles between TGF-beta treated and untreated cells, which could be explained by linked SNPs in cis-regulatory elements. We are now comparing these results to ChIP-Seq data gathered under the same conditions to understand the relationship between allele-specific binding of transcriptional regulators and allele-specific expression of nearby genes.

Genome-wide DNA methylation screening in breast cancer revealed new candidate loci potentially involved in field defect

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DNA methylation analysis is considered a promising tool for cancer diagnosis. We screened breast tumors and corresponding patient-matched normal-appearing tissues for DNA methylation patterns using Illumina infinium platform. Additionally, we added normal breast tissue samples from cancer-free individuals to the screening panel as control. 1039 CpGs were differentially methylated between tumors and normal tissues from cancer-free people. Among these, we identified a signature consisting of 678 CpGs that could distinguish between non-cancer and cancer breast cell lines based on their level of methylation. Most of these CpGs have almost the same respective degree of DNA methylation in samples from healthy individuals and normal tissues from cancer patients. However, we identified several CpGs with different methylation levels between normal breast tissues from cancer patients and healthy individuals. Interestingly, the methylation patterns of these loci in control samples from cancer patients show a tendency to the patterns observed in tumor samples as compared to the normal tissues from healthy people, suggesting the possible involvement of these loci in breast cancer field defect. Gene-ontology analysis categorized most of the genes into the functional groups associated with cell death, cell cycle, cellular assembly and organization, cancer, endocrine system development, gene expression and genetic disorders.

Galectin-4 upregulation is associated with -233 point mutation and hypomethylation in colorectal cancer

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Background and Methods:
Colorectal cancer (CRC) is one of the most common cancers among men and women and accounts for 10% of all new cancer cases and cancer deaths each year. Seems that cancer is not only attributed to deregulations of multiple genes genetically and epigenetically, but also many genetic and epigenetic changes are behind the regulation of a single gene. In the present study, we profiled the expression pattern of different stages of six colorectal cancer and adenoma cell lines (SW1116, SW480, SW620, Co115, KM20L2, and LT97) in comparison to normal colon cell line CCD-18co. Among the regulated genes, we focused to understand the underlying mechanism of Galectin-4 upregulation in two out of six cell lines (LT97 and KM20L2). The microarray results have been validated using qRT PCR and Western blot. The methylation status has been detected by bisulfite sequencing. In parallel, the -400 to +50 subjected to sequencing and subsequently the wild sequence and the mutant sequence has been cloned in luciferase reporter vector and transfected to SW620 and Co115 cell lines. In order to investigate whether the detected sequence change is mutation or single nucleotide polymorphism (SNP), we have screened the tumors of 12 colorectal cancer patients and their adjacent normal tissue.

Results:
Galectin-4 is upregulated in two out of sex cell lines (LT97 and KM20L2) using the microarray technology and qRT PCR. Moreover, we have detected upregulation in the protein level using Western blot. Using the bisulfite sequencing of the 1st exon and upstream sequence, we have found hypomethylation in two cell lines (LT97 and KM20L2). Interestingly, in LT97 and KM20L2, a point mutation at -233 has been found. A 1.5 fold change of luciferase activity in the mutant clone. Among the 12 colorectal cancer patients, two out of twelve showed -233 point mutation in the tumor DNA but not in the adjacent normal tissue.

Epigenetic alterations in a mouse model of intestinal tumor formation

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Mutations in the APC gene (adenomatosis polyposis coli) lead to a deregulation of the Wnt/beta-Catenin signalling pathway and are common in hereditary and sporadic cases of human colorectal cancer. Epigenetic changes like DNA hypermethylation in CpG rich promoters are a common feature of neoplasias and play a major role during tumor formation and progression. In order to gain insight into the DNA-methylation alterations associated with tumor formation, we are analyzing DNA-methylation in the Apc-min/+ mouse, a model of the early steps of intestinal cancer. DNA methylation is analysed by methylated DNA immunoprecipitation followed by second generation sequencing (MeDIP-Seq), allowing in principle an unbiased genome wide analysis. We will present data on DNA methylation patterns and RNA expression from tumor and normal tissue of the Apc-min/+ mouse model, revealing epigenetic changes occurring during the early steps of tumor formation.

National Genome Research Network

Poster presentation abstracts

Symposium III

Animal, Cellular and Tissue Models

Effects of Uremic Mediators on Activated Endothelial Cells

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Accumulation of uremic retention solutes during loss of kidney function promotes uremic cardiovascular disease (CVD), a major cause of mortality in patients with chronic kidney disease (CKD). So far, more than 100 uremic toxins have been identified, so far.

To gain insight into the biological mechanisms of specific uremic toxins and renal mediators, a large number of uremic toxins was analysed for their effects on vascular cells, such as cell proliferation and apoptotic functions. To consider the hemodynamic environment in the arterial system, we established an in vitro co-culture model, in which human primary endothelial cells can be exposed to different shear stress levels, mimicking physiological but also patho-physiological shear stress conditions. This cell culture model was used to further analyse specific uremic mediators and renal vascular regulators for their molecular mechanisms and vascular function. Secretome analysis using MALDI-TOF/TOF mass spectrometry was performed, showing that a dinucleoside polyphosphate was released by activated endothelial cells, but not by smooth muscle cells in our hemodynamic co-culture model. This vasoconstrictive dinucleoside is known to be produced and secreted by renal tissue, affecting pre-glomerular arterioles. However, the mechanism still needs to be further elucidated.

Aim of the study is, to characterize molecular function of specific uremic mediators in order to identify putative biomarkers for diagnosis and prediction of cardiovascular effects in CKD in the context of the NGFN Transfer Project “New Tools for the Prevention of CVD in CKD”.

Low salt diet induced expression of kidney injury and degeneration biomarkers

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New tools for the detection of the progression of chronic kidney disease (CKD) or chronic cardiovascular disease (CVD) include the identification of early and sensitive biomarkers that are correlated with pro-fibrotic or pro-inflammatory processes that ultimately result in tissue remodeling, loss of tissue function and end organ degeneration. In order to identify highly responsive and sensitive biomarker candidates for early remodeling and stress-related processes, a rodent model was used to monitor the expression profiles in kidney and heart tissues under very mild physiological changes of osmolality and endogenous aldosterone regulation due to a diet with a shift to reduced sodium chloride content (0,02% w/w NaCl). Longitudinal gene expression profiles were determined by Affymetrix gene chip and RTPCR analyses in renal cortex and myocardium after 3 days, 5 days, and 10 days versus control groups (0,2% w/w NaCl). A series of genes were transiently induced by the shift to the low salt diet, other genes were significantly induced only after a prolonged period of low salt diet application corresponding to increased plasma aldosterone levels. Notably, gene products referred to as kidney injury or degeneration markers were induced very pronounced under these gentle conditions on day 3 and day 5, e.g. KIM-1 (HAVCR1), NGAL (LCN2), osteopontin (SPP1). Gene products of the renin – angiotensin – aldosterone system (RAAS) were also recorded for control. These preclinical findings are being evaluated in a translational medicine approach for biomarker and therapeutic target qualification employing samples from CKD or chronic CVD patients in the context of the NGFN Transfer Project “New Tools for the Prevention of CVD in CKD”

Regulation of Podoplanin expression in primary gliomblastoma

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The oncogenic transcription factor AP-1 is mainly composed of Jun and Fos proteins and mediates gene transcription in response to a plethora of extracellular and oncogenic stimuli. Expression and function of AP-1 and its downstream target genes are critically involved in neoplastic transformation as well as malignant progression.
In a study performed within the Brain Tumor Network Plus (BTNplus) on the expression profile of genes in astrozytic glioma samples compared to normal brain we found high expression of the Fos-target gene podoplanin (PDPN). Interestingly, increased PDPN transcript levels were specific for primary glioblastoma multiforme (GBM) and negatively correlated with the expression of PTEN, functional loss of which is an early event in primary GBM.
In order to define the regulatory pathways leading to induction of PDPN expression, we we analysed the expression of PDPN in different human glioma cell lines. Cell lines harbouring in PTEN mutation (LN308, LN319, U87MG, and U373MG) were positive for PDPN, whereas all cell lines containing wild-type PTEN (LN18, LN229, LN428) were PDPN-negative both on RNA and protein levels. Reintroduction of wild-type PTEN in the PTEN-mutant cell lines, pharmacological inhibition of the PTEN downstream target protein kinase B/Akt as well as interference with the function of Jun and Fos resulted in a reduction of PDPN expression. Taken together, we propose a model of PDPN regulation in human glioblastoma cells, involving increased PI3 kinase activation mediated by loss of PTEN function, resulting in subsequent activation of Akt and AP-1 and, thus, an increase in PDPN transcript and protein. Our data indicate that targeting the activation of AP-1 and/or its downstream target Podoplanin could be a promising strategy for the treatment of patients with primary glioblastoma.

In vivo investigation of the transcriptional co-activator CITED4 and its role in glial tumourigenesis

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Oligodendroglioma are primary tumours of the brain parenchyma. A genetic hallmark of these tumours is the combined loss of chromosomal arms 1p and 19q (LOH 1p19q) which is correlated with a better response to chemo- and radiotherapy and thereby with a prolonged survival of the patients. Therefore, pathophysiological relevant genes were presumed to be located in this genomic region. In previous studies based on gene expression profiling comparing 1p19q LOH-tumours vs. tumours with 1p19q retention (Ret) we demonstrated significant downregulation of CBP/P300-Interacting Transactivator with ED-rich tail 4 (CITED4), located on 1p34, to be downregulated in the LOH cases. CITED4 downregulation is not only caused by haploinsufficiency due to genomic loss but is also mediated by promoter hypermethylation of the residual allele. CITED4 has been described to be a transcriptional co-activator of the transcription factor AP2 and to negatively regulate HIF1alpha-dependent gene expression.
In order to address its in vivo function a constitutive CITED4 knockout mouse was generated. Homozygous knockout animals are viable and fertile. A comprehensive phenotypic analysis is currently conducted in collaboration with the German Mouse Clinic (GMC).
To study the role of CITED4 in glial tumorigenesis, we will apply a murine oligodendroglioma model based on somatic gene transfer using the RCAS/Tva system. CITED4 overexpression or knockout, respectively, combined with PDGF-driven tumour induction will be followed by survival analysis as well as evaluation of tumour histology and MRI-based contrast enhancement. Additionally, gene expression profiling of CITED4 modulated vs. control tumours will help to integrate the data into a biological model.
Taken together, this approach will be the first investigation of Cited4 in vivo and will give novel insights into the role of CITED4 in glial tumourigenesis.

Identification and validation of differentially expressed proteins associated with hypoxia in human malignant glioma cell lines

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The most common type of primary malignant brain tumors are glioblastomas. These highly aggressive, rapidly growing tumors are exposed to hypoxia which occurs as a consequence of inadequate blood supply. Proteomics techniques are employed to detect changes in the overall protein pattern of malignant glioma cell lines after hypoxia treatment compared to normoxic controls.

LNT229 glioblastoma cells were exposed to hypoxia (1% oxygen) or normoxia for 72 hours. Total cell lysates were separated by high-resolution 2D gel electrophoresis followed by silver staining or 2D DIGE experiments. Differentially expressed proteins were identified by MALDI TOF and ESI QTof mass spectrometry. For validation of deregulated proteins Western blotting was carried out with protein lysates from LNT229, U87 and LN18 glioma cell lines after 0h, 8h, 24h and 72h of hypoxia/normoxia treatment.

The human malignant glioblastoma cell line LNT229 was initially used in our studies to detect hypoxia associated, differentially expressed proteins in cell lysates from cells grown under hypoxic and normoxic conditions for 72 hours. Differential analysis was performed using high-resolution 2D electrophoresis. By image analysis, 13 differentially expressed protein spots were detected in analytical 2D gels and could be identified by mass spectrometry. To evaluate these deregulated proteins we are currently investigating by Western blot analysis their expression in three different glioma cell lines (LNT 229, U87 and LN18) and at four time points (0h, 8h, 24h and 72h) of hypoxia/normoxia treatment. Up to now, two proteins could be confirmed as upregulated and one protein as downregulated after hypoxia treatment in all three cell lines. These proteins will be further evaluated in Western blots of cell lysates from human glioma-derived primary cell cultures.

Irradiation-enhanced mammalian target of rapamycin (mTOR)-targeted glioblastoma therapy with CCI-779 (temsirolimus)

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The phosphatidylinositol 3-kinase (PI3K)/Protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway plays a critical role in oncogenesis and dysregulation of the pathway is common in human malignant gliomas. In these tumors, activation of PI3K/Akt/mTOR signaling leads to cell cycle progression, neovascularization, and escape from apoptosis, and is associated with poor prognosis. CCI-779 (Temsirolimus) is a small-molecule inhibitor of the mTOR kinase that has been demonstrated to have some antiglioma activity. Given that postoperative radiochemotherapy is the standard of care in the first-line treatment of glioblastoma, this translational project aims at analyzing whether CCI-779 improves the efficacy of irradiation in experimental glioma models and further to clarify the impact of PTEN in mTOR inhibition. In different human malignant glioma cell lines, we observed that CCI-779 applied at effective but not yet immunosuppressive concentrations exerted marked antiproliferative, antiangiogenic, anticlonogenic and proautophagic activity that was enhanced through irradiation. These effects were independent from the PTEN status, and were paralleled by a feedback loop activation of Akt. Moreover, CCI-779 when applied following radiosensibilization, inhibited glioma invasiveness in a supra-additive way and reverted the proinvasive effect of sole sublethal irradiation. In a MRI-monitored syngeneic, orthotopic mouse glioma model, combined treatment of CCI-779 and irradiation demonstrated antitumoral and antiangiogenic activity and prolonged survivial significantly compared with irradiation- or CCI-779-treatment alone.
Further ongoing in vitro investigations focus on effects in glioblastoma-initiating cells, cRNA microarray analyses, and validation of downstream candidates. Taken together, the results support the clinical evaluation of combined targeted mTOR inhibition with CCI-779 and radiotherapy in patients with newly diagnosed glioblastomas.

IG Cellular Systems Genomics – Network analysis of tumor drug resistance

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The IG-Cellular Systems Genomics combines high-throughput reverse genetic screening approaches with quantitative proteomics 1 and cell biology 2 technologies to unravel mechanisms of drug resistance in cancer treatment 3. Our initial focus is on breast cancer, where the ERBB-signaling network is known to be causally related to disease progression 4. We perform systematic screening for novel components of relevant signaling pathways and validate findings via protein interaction mapping. Since the observed cross-talk between individual signaling pathways is not only via protein interactions, we extend on the impact miRNAs 5, epigenetic changes and mutations have in order to also cover longer term effects. The comprehensive analysis of ERBB-signaling, initially in cell-line models and then in patient samples, will give us an integrated view on the impact this signaling network has in breast cancer, and lay the ground for a better understanding of the molecular mechanisms leading to disease, resistance and metastasis. This directs us towards systems genomics models that predict and validate novel markers for diagnosis and prognosis as well as target molecules with potential for therapeutic intervention.

For more information visit http://www.igcsg.org

1 Lobke, C. et al., Proteomics 8 (8), 1586 (2008).
2 Sauermann, M. et al., Oncogene 27 (9), 1320 (2008).
3 Sahin, O and Wiemann, S, FEBS Lett. 583 (11), 1766 (2009).
4 Sahin, O. et al., BMC Systems Biology 3, 1 (2009).
5 Haller, F. et al., J Pathol, accepted (DOI: 10.1002/path.2610) (2009).

Phenotypic characterization of the Vacuole membrane protein 1 knockout mouse

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Vacuole membrane protein 1 (Vmp1) is a plasma membrane protein, which plays an essential role in initial cell-cell contacts in vitro. Vmp1 interacts with the tight junction protein ZO-1 and co-localizes in spots between neighboring cells. In addition, it is temporally expressed during adherent junction formation. Downregulation of Vmp1 by RNAi results in loss of cell adherence, and increases the invasion capacity of a non-invasive kidney cancer cell line. In the context of cancer, VMP1 mRNA level is significantly reduced in kidney cancer metastases as compared to primary tumors.
To characterize the function of VMP1 in vivo, a mouse model targeting the VMP1 gene both constitutively and conditionally was developed. Breedings between constitutive heterozygous VMP1+/- knock out mice failed to yield homozygous VMP1-/- knockout mice. Next, using mice expressing tamoxifen inducible Cre, VMP1flox/flox was deleted by intraperitoneal administration of tamoxifen for five consecutive days. Twelve hours after the fourth tamoxifen injection, VMP1flox/floxCre+ mice display ataxia, paleness of liver and colon, as well as significant ascitic fluid. This phenotype leads to death within few hours. In future, further studies on VMP1 knockout mice in the context of tissue integrity and motor coordination will contribute to understand its function in vivo.

Quantification and molecular characterization of circulating tumor cells in breast cancer patients

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The existence of circulating epithelial tumour cells in the blood of cancer patients has been known for a long time. In recent years, advanced technology enabled several studies, detecting circulating tumour cells (CTCs) during an onward tumour development and suggests a prognostic and predictive role in metastatic disease, as well as in adjuvant treatment.
The objective of this project is to test if CTCs are detectable at the time-point of initial diagnosis of breast cancer, and how the occurrence of CTCs during the follow-up of the same patients correlates with tissue-based tumour markers, lymph node status and applied therapies. In addition, we aim to characterize CTCs concerning their expression of prognostic markers such as HER2, breast mucin (MUC1) and CD44 glycoproteins. Overall, we will analyse 800 blood samples at the time-point of biopsy, and approximately 150 clinical follow-ups (every six month) of patients included in this project.
Initially, we set up the detection of epithelial cells by using a model system in which 10 to 1000 breast carcinoma cells were diluted into blood samples from healthy donors. Then, cells were enriched by high–gradient magnetic cell sorting (MACS), stained with specific antibodies against epithelial markers and detected via flow cytometry. Applying this method to 30 ml blood samples, taken at the time-point of biopsy, we detected between 1 to 18 cytokeratin-expressing tumour cells in the blood of 21% of the patients tested so far (33).
In future, we will also use fluorescence microscopy-based methods for the analysis of CTCs to confirm our previous findings, and to expand the detection of cell surface markers. We suppose that our results will aid in the prognosis for metastatic relapse, as well as in monitoring the effectiveness of different breast cancer treatments.

Generation and characterization of a trastuzumab resistant breast cancer cell line to test combinatorial treatments with HER2- and EGFR inhibitors

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Trastuzumab (Herceptin®) is a recombinant humanized monoclonal antibody against the extracellular domain of the human epidermal growth factor receptor 2 (HER2). Currently, trastuzumab is the only HER2-targeted therapy approved by the FDA for adjuvant treatment of early breast cancer and first-line treatment of HER2-positive metastatic breast cancer. The her2 gene is overexpressed in 20-25% of invasive breast cancers and associated with poor survival and resistance to certain chemotherapeutic agents. The majority of HER2 positive patients initially respond to trastuzumab. However, relapse is common and a serious challenge in the treatment of patients with HER2 overexpressing breast cancer.
The aim of this project is to identify mechanisms involved in acquired trastuzumab resistance in vitro, and to evaluate new drug combinations with the HER2 targeting antibody pertuzumab and the EGFR inhibitor erlotinib. For this purpose, we started to establish a resistant cell line using the trastuzumab sensitive breast cancer cell line BT-474. The resistant cell line BT-474tr was treated with trastuzumab, pertuzumab and erlotinib, alone or in combinations. As expected, the BT-474tr cells failed to undergo cell cycle arrest when treated with trastuzumab alone and showed a similar growth curve compared to untreated control cells. The same was observed for pertuzumab treated cells, whereas cells treated with erlotinib showed a clear arrest in the G1-phase. Interestingly, cells treated with the combination of erlotinib and pertuzumab showed a synergistic effect compared to erlotinib alone or in combination with trastuzumab.
Using this strategy, analysis of cell cycle, mRNA- and protein profiles from cells treated with increasing concentrations of trastuzumab will be performed to model the mechanisms leading to trastuzumab resistance. Finally, the data will be utilized to define novel targets for combinatorial treatments to avoid acquired resistance in HER2 positive breast cancer.

Pharmacological treatment during the neonatal period improves neurological sequelae in a transgenic mouse model for familial neonatal convulsions

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The mechanisms of epileptogenesis in the neonatal brain are only partially understood. Consequently, there are no antiepileptic treatment strategies specifically adapted to the physiology of the neonatal brain. To explore novel antiepileptic strategies for neonates, we generated a mouse model for benign familial neonatal convulsions (BFNC) that lacks functional KCNQ potassium channels by Tet-Off system-mediated overexpression of dominant-negative KCNQ2-subunits in neurons. Suppression of KCNQ channels during the neonatal period resulted in a severe phenotype including neurodegeneration, spontaneous epilepsy, and marked behavioral changes in adult mice, whereas absence of transgene expression during the first two neonatal weeks prevented this phenotype.

As the GABAergic neurotransmitter system plays a key role in early network development, we tested for the effects of the NKCC1 blocker bumetanide and the GABA receptor antagonist picrotoxine in neonatal mice. Acute treatment with these drugs or with the KCNQ channel opener retigabine reduced cortical and hippocampal network activities in vivo. In transgenic animals lacking functional KCNQ channels, chronic pharmacological treatment with either drug during the first two neonatal weeks reduced morphological hippocampal changes and improved the behavioral phenotype of adult animals.

Our data suggest that the control of network oscillations by cortical KCNQ channels is critical in the neonatal brain. Drug treatment during this early period might provide an effective treatment option for hyperexcitable neonatal networks in general.

Sodium channel accessory subunits determine antiepilptic drug sensitivity via paradoxical effects on persistent sodium currents

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Neuronal excitability is critically determined by the properties of voltage-gated Na+ currents. Na+ channels are composed of a pore-forming alpha subunit and one or two accessory beta subunits that are known to modify the biophysical properties of pore-forming subunits. It is well-established that mutations in genes encoding accessory subunits of voltage-gated Na+ channels can give rise to epilepsy.

We have examined the idea that the presence of beta subunits also modifies the pharmacological properties of the Na+ channel complex using mice lacking either the beta1 (Scn1b) or beta2 (Scn2b) subunit. We examined effect on fast transient Na+ currents (INaT), which mediate the fast upstroke of action potentials, and on low voltage activated persistent Na+ currents (INaP) that contribute to subthreshold excitation. Classical effects of the anticonvulsant carbamazepine (CBZ), such as use-dependent reduction of Na+ channel availability and effects on the voltage-dependence of inactivation, were unaltered in mice lacking beta subunits. Surprisingly, we found that CBZ induced a small but significant shift of the voltage-dependence of activation of INaT and INaP to more hyperpolarized potentials. This novel CBZ effect on INaP was strongly enhanced in both Scn1b and Scn2b null mice. This effect led to a pronounced increase of INaP at subthreshold potentials in Scn1b null mice. Importantly, this effect led to a complete loss of CBZ efficacy in reducing repetitive firing in these mice.

Thus, beta subunits modify not only the biophysical but also the pharmacological properties of Na+ channels, in particular with respect to INaP. These results suggest that altered beta?subunits in neurological disorders may cause altered neuronal sensitivity to drugs targeting Na+ channels.

Homeostasis of the spike afterdepolarization in CA1 pyramidal neurons

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Pyramidal neurons in the central nervous system express a multitude of voltage-gated ion channels that determine the neuronal response to synaptic stimulation. Thus, the ability to maintain a constant stochiometry of different types of channels is essential to ensure stability of neuronal integrative properties over time. Accordingly, homeostatic mechanisms have been proposed to exist that maintain an appropriate firing behavior in the face of continuous ion channel turnover. Understanding these mechanisms is of considerable importance, in particular when evaluating the neuronal response to the presence of loss- or gain-of-function mutations in ion channel genes.

We have focused on the homeostatic regulation of two conductances that affect neuronal excitability in opposite ways. Activation of the persistent Na+ current (INaP) augments action potential afterdepolarizations (spike ADPs) and bursting behavior, while activation of M-type K+ currents (IM) have the converse effect. Intriguingly, both channel proteins show a co-localized aggregation at the axon initial segment.

An examination of the discharge behavior in mice lacking functional Nav1.6 channels (Nav1.6-null MED-mice) revealed that spike ADPs were unchanged, even though INaP was reduced by 40%. In contrast, pharmacologically blocking INaP acutely, decreases the spike ADP. As this finding suggested homeostatic co-regulation of ion channels, we have established an electrophysiological protocol that allows us to measure INaP and IM in the same CA1 hippocampal pyramidal neuron. We found that a ~40% decrease in INaP in MED-null mice is accompanied by a similar down-regulation of IM (~30%).

Collectively, these findings suggest that chronic, but not acute reductions in INaP lead to a specific down-regulation of IM consistent with a homeostatic regulation. These findings, and the molecular mechanisms underlying them, are likely to be relevant for understanding the impact of mutations in ion channel genes.

Human embryonic stem cell-derived neurons exhibit functional amyloidogenic APP processing that can be modulated by familial AD-associated mutations of the presenilin-1 gene.

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Alzheimer's disease (AD) is the most frequent cause for dementia, characterized by a progressive neuronal loss. Compelling evidence indicates that accumulation of the amyloid-beta (Aß) protein that forms extracellular plaques in the brain plays a causal role in the development of AD. Here, we characterize a postmitotic neuronal culture system generated from human embryonic stem cell-derived neural stem cells (lt-hESNSC). These cells endogenously express the human major AD-associated proteins including amyloid precursor protein (APP) as well as the ß- and ?-secretases involved in its amyloidogenic processing. We demonstrate efficient neuronal differentiation of lt-hESNSC and generation of Aß from endogenous APP. Modulation of amyloidogenesis was assessed by lentiviral over-expression of the ?-secretase component presenillin-1 (PS1) including its L166P mutation that causes early onset familial AD in humans. We show that the L166P mutation causes a loss-of-function leading to decreased cleavage of APP-CTFs and secreted Aß40 and an increase in Aß42/Aß40 ratio in the extracellular space. Thus, wildtype and PS1-transgenic hESNSC should represent a valuable tool to assess AD-related cytophathological processes, thereby circumventing major problems of heterologic expression systems.

DiGtoP: From Disease Genes to Protein Pathways

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The DiGtoP network aims at systematically and comparatively investigating protein interactions for several disease-associated genes in man and mouse.
Substantial progress has been made in the identification of gene mutations associated with certain diseases as e.g. neurological and psychiatric disorders, cancer, diabetes, and cardiovascular diseases.
The actual challenge is to determine how these mutated genes and their underlying protein networks affect the development of the respective disease.
Recent technical advances made it possible to reliably determine interactions within the mammalian proteome. Particularly, the proteome mapping approach offers a way forward by identifying physical protein relationships and by indicating pathways which then can be validated by functional analysis in vitro and in vivo. The choice of the appropriate experimental model is crucial as protein interactions are dependent on time and cell type.
DiGtoP analyzes the core proteome in vitro by using HeLa cells as well as human and mouse embryonic stem (ES) cells. The possibility to differentiate ES cells into any cell type, mimicking embryogenesis in vitro, offers the opportunity to compare signal transduction and protein interaction of disease-associated genes in parallel in mouse and man.
The DiGtoP consortium uses a modified GFP tag to document the sub-cellular localization of proteins in vitro and in vivo and to determine their interaction partners by affinity chromatography and mass spectrometry. This approach, complemented by functional validation of the determined interactions via siRNA technology and knock-out mouse models, leads to the establishment of a mammalian proteome database of unrivalled quality that is relevant for the understanding of disease pathways. Furthermore, DiGtoP represents a pipeline well designed for the study of any novel disease genes.

Regulation of Astrocyte Inflammatory Responses by the Parkinson's Disease-Associated Gene DJ-1

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The Parkinson's disease (PD)-associated DJ-1 mediates direct neuroprotection. The up-regulation of DJ-1 in reactive astrocytes also suggests a role in glia. Here we show that DJ-1 regulates pro-inflammatory responses in mouse astrocyte-rich primary cultures. When treated with a toll-like receptor 4 agonist, the bacterial endotoxin lipopolysaccharide (LPS), Dj-1 knockout astrocytes generated >10 times more nitric oxide (NO) than littermate controls. Lentiviral reintroduction of DJ-1 restored the NO response to LPS. The enhanced NO production in Dj-1-/- astrocytes was mediated by a signalling pathway involving reactive oxygen species leading to specific hyper-induction of type II NO synthase (iNOS). These effects coincided with significantly increased phosphorylation of p38 mitogen-activated protein kinase (MAPK), and p38MAPK inhibition suppressed NO production and iNOS mRNA and protein induction. Moreover, enhanced phosphorylation of p38MAPK was detected in DJ-1 deficient Caenorhabditis elegans exposed to gram-negative bacteria. Dj-1-/- astrocytes also induced significantly stronger the pro-inflammatory mediators cyclooxygenase-2 and interleukin-6, but not nerve growth factor. Finally, primary neuron cultures grown on Dj-1-/- astrocytes became apoptotic in response to LPS in an iNOS-dependent manner, directly demonstrating the neurotoxic potential of astrocytic DJ-1 deficiency. These findings identify DJ-1 as a regulator of pro-inflammatory responses and suggest that loss of DJ-1 contributes to PD pathogenesis by deregulation of astrocytic neuroinflammatory damage.

Calpain Cleavage of a-Synuclein in the Pathogenesis of Parkinson´s disease: In vivo Analysis of Double Transgenic Mouse Models

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Parkinson´s disease (PD) is a common neurodegenerative and slowly progressive disorder. Mutations and gene multiplications of the alpha-synuclein gene are known to cause familial PD. Alpha-synuclein is a small soluble protein and it is expressed primarily at presynaptic terminals throughout the brain. The precise function ofalpha-synuclein is unknown so far. However, it is also a major protein component of the neuropathological hallmarks of PD, so-called Lewy bodies (LBs). LBs are cytoplasmic inclusion whose primary structural component is insoluble and fibrillized alpha-synuclein. Mechanisms by which alpha-synuclein switches conformational state, accumulates and becomes toxic to especially dopaminergic neurons of the substantia nigra are still unknown.

Calpain is a calcium-activated protease and alpha-synuclein is a substrate for calpain cleavage. Calpain cleaved alpha-synuclein species are similar in molecular weight to truncated alpha-synuclein fragments that promote alpha-synuclein aggregation and enhance cellular toxicity.

Therefore, our study is focused on the functional implication of calpain-mediated proteolytic cleavage of alpha-synuclein in the pathogenesis of PD. Calpastatin is the only natural inhibitor of both, µ- and m-calpains In order to analyze impact of calpain-cleaved alpha-synuclein, we generate and characterize two contrasting mouse models, expressing human mutated [A30P] alpha-synuclein either in mice overexpressing human calpastatin or on a calpastatin-deficient background. Analysis of double transgenic mice will reveal, whether inhibition or increased activation of calpain leads to alterations of a SNCA induced phenotype in this PD mouse models.

Function of BACE1 and Neuregulins in the developing and adult nervous system

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BACE1 (beta-site APP cleaving enzyme), an aspartyl protease, plays a critical role in the production of amyloid Abeta peptides; insoluble plaques containing Abeta constitute the molecular basis of pathogenesis in Alzheimer's disease. One principal physiological function of BACE1 is the cleavage of Neuregulins, EGF-like growth factors that are highly expressed in the nervous system. Previously it has been shown that BACE1 is required for cleavage of axonally-expressed type III Neuregulin-1, and that BACE1 activity is essential for peripheral nerve myelination, a process that depends on type III Neuregulin-1 signaling to apposing Schwann cells. We have now generated mice carrying compound mutations in BACE1 and specific isoforms of Neuregulin-1 with the aim of further characterizing the functions of BACE1 in Neuregulin-1 signaling in the neonatal and adult mouse nervous system. As well as revealing genetic synergy between BACE1 and type III Neuregulin-1 in peripheral nerve myelination, analysis of compound mutant mice has shown that BACE1 is also necessary for the function of other Neuregulin-1 isoforms. Behavioral studies of compound mutant mice are currently under way and could help to shed light on the role of Neuregulin-1 in the development of schizophrenia and psychosis.

Statistical analysis and modeling of rodents alcohol drinking time series.

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Time series from different phases(first exposure, conditioning and baseline behavior) of alcohol intake in rodents are statistically analyzed in order to:
- Find different patterns of behavior in each phase,
- Correlate patterns of different phases
- Modeling and prediction of animal baseline drinking behavior from early intake exposures.

1H MRS profiling at 9.4 T in medial prefrontal cortex and hippocampus of ethanol dependent rats during intoxication, withdrawal and protracted abstinence

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The key clinical problem in alcoholism is relapse into excessive drinking, but biomarkers for predicting an individual's vulnerability to relapse are lacking. Experimental alcohol vapor intoxication of laboratory animals allows emulating a level, pattern and duration of brain exposure that shares key features with what occurs in clinical alcoholism. Thus, chronic intermittent alcohol intoxication of rats leads to persistent neuroplasticity even after prolonged periods of abstinence and changes in behavioral responses, similar to key symptoms seen in alcoholics.

Here, we assessed metabolic profiles in medial prefrontal cortex (mPFC) and hippocampus (HI) using in vivo single-voxel 1H magnetic resonance spectroscopy on a 9.4 T scanner. Rats were made dependent by exposure to 45 daily exposure cycles with peak levels up to 4 g/l blood alcohol concentration. Five MRS recordings covering ethanol intoxication, acute withdrawal and protracted abstinence were obtained in a within subject, repeated measurement design. Spectra were acquired using Point Resolved Spectroscopy (PRESS) at an echo time of 10 ms from mPFC and HI volumes (12 and 16 µl, respectively). LCMODEL was used for quantification by fitting the in vivo spectra to phantom data of 16 different metabolites. Concentration values were referenced to a non-suppressed water signal acquired from the same voxel.

Preliminary results show reduced myoinositol and creatine/phosphocreatine levels as well as increased total choline-containing compounds during intoxication. Raised glutamate levels are found during early withdrawal. With the exception of taurine, all metabolites returned to control levels after 3 weeks of abstinence.

These results confirm findings from human alcoholics and thus demonstrate the validity of our experimental approach for studying mechanism associated with the pathophysiology of alcoholism, and potentially for monitoring the efficacy of pharmacotherapeutic interventions.

Conditional ablations of glutamate receptors in dopaminergic and dopaminoceptive neurons

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The persistent and recurring nature of addiction is associated with glutamate-dependent plasticity of neurons within the mesocorticolimbic system of the brain. Drugs modify the excitatory synapses on midbrain dopaminergic as well as forebrain dopaminoceptive neurons, presumably by NMDA receptor-dependent synaptic incorporation of GluR1-containing AMPA receptors. In order to dissect the roles of specific glutamate receptors in development of persistent drug-dependent behaviors, we generated mice with specific, Cre/loxP mediated ablations of genes encoding GluR1, GluR2, or NR1. Stable and reproducible patterns of target gene recombination in the dopaminergic or dopaminoceptive cells were achieved with transgenes were Cre encoding sequence is introduced at the translation start of BAC-derived fragments of the DAT and D1 genes respectively. Furthermore, we have generated mice expressing the tamoxifen-inducible Cre fusion protein (CreERT2), allowing for temporal control of the mutation onset. By use of the DATCreERT2 and D1CreERT2 strains we can not only exclude any developmental impairments, but most importantly distinguish the roles of glutamate receptors in acquisition, persistence and relapse of drug-induced behaviors.

ZF125 mutations destabilize cardiac Z-disks and lead to dilated cardiomyopathy

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Z-disks are the mechanical integration sites of heart and skeletal muscle cells linking anchorage of myofilaments to force reception and processing. Key-molecules that enable the Z-disk to persistently withstand the extreme mechanical forces during muscle contraction have not been identified yet. Here, we isolated ZF125 as a novel Z-disk protein. To analyze its physiological function we inactivated ZF125 in zebrafish by morpholino-modified antisense RNA mediated gene-knockdown. Loss of ZF125 leads to perturbed Z-disk stability and consecutively to heart failure. To evaluate the role of ZF125 in human heart failure, we performed a genetic association study on dilated cardiomyopathy (DCM) patients and find several ZF125 mutations associated with DCM. ZF125 mutation carriers show the same cardiac Z-disk pathology as observed in ZF125-deficient zebrafish. Expression of the human ZF125 mutations in zebrafish induces Z-disk damage and heart failure, indicating a dominant-negative effect and confirming their disease-causing nature. Increasing mechanical strain aggravates Z-disk damage in ZF125-deficient heart and skeletal muscle, implying a unique role in protecting Z-disks from mechanical trauma.

Overexpression of an Abcc6-encoding protein variant from C3H/He enhances calcification in mesenchymal stem cell

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Abcc6 belongs to a gene family encoding for ABC-transporters with an ATP-binding cassette. In mice, Abcc6 was found to predispose C3H/He strains to dystrophic cardiac calcification (DCC). The sequence of Abcc6 is found to vary in seven base pairs leading to amino acids exchanges between DCC-resistant C57BL/6 and DCC-susceptible C3H/He mice.
The aim of this study is to study the role of these variants in calcification in an in vitro cell culture model.
Both variants of cDNA encoding for the C3H/He and the C57BL/6 protein were cloned and inserted in a pSG5 expression vector (pSG5-Abcc6-C3H and pSG5-Abcc6-B6 respectively). We established a calcifying cell culture model using the mesenchymal stem cell line C3H10T1/2. Calcification was induced adding inorganic phosphate to the media. Cells were transfected with empty pSG5 vector (pSG5), pSG5-Abcc6-C3H and pSG5-Abcc6-B6. Cells were analyzed 3, 7 and 21 days after induction of calcification. Calcium deposits were stained using Alizarin Red S staining and quantified by Randox Ca Kit.
Generally, the time course analysis after induction of calcification revealed no calcification after 3 days, an initiation of calcification after 7 days and a strong calcification after 21 days. Seven days after transfection, the mean values of calcium deposits were measured to be 9.42 µmol in pSG5 empty vector, 13.76 µmol in pSG5-Abcc6-C3H and 9.13 µmol in pSG5-Abcc6-B6 transfected cells (n=3x3). A significant increase of 1.55-fold in calcium deposits was found in cells transfected with pSG5-Abcc6-C3H compared to those transfected with pSG5 (13.75 vs 9.42, respectively; p=0.0063). However a non significant decrease in calcium deposits was observed in cells transfected with pSG5-Abcc6-B6 compared to pSG5 (9.13 µmol vs 9.42 µmol respectively; p=0.7321).
Using cell culture model, we functionally demonstrate for the first time the effect of the amino acid substitutions found in the C3H/He-Abcc6 on calcification in vitro.

MRas - A Strong Candidate Gene (SCG) for Coronary Artery Disease (CAD)

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In our recent genome-wide association study (GWAS) we identified the MRas gene as a SCG for CAD. M-Ras is a member of the Ras superfamily of small GTPases, thes family members function as molecular switches in diverse cellular functions and thereby regulate a variety of biological processes. Atherosclerosis is a chronic inflammatory disease of the vessel wall with accumulation of lipid-laden macrophages in the large arteries. RT-PCR analyses demonstrated that MRas is expressed in several tissues, including mouse and human aorta and heart, tissues that are involved in atherosclerosis. M-Ras has been implicated in the regulation of TNFa-stimulated LFA-1 activation and integrin-mediated leukocyte adhesion downstream of various inflammatory cytokines. M-Ras could thus be linked to monocyte adhesion onto the endothelium. We have obtained MRas-KO mice and we are currently crossing MRas-null mice with Apolipoprotein E (ApoE)-KO mice in order to create double-KO animals because Apoe-KO mice are highly susceptible to atherosclerosis. We will perform a mouse-atherosclerosis study.

Large-scale phenotyping of EMP3-knockout mice at the German Mouse Clinic reveals a specific role of the gene in immunity

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Background: Epithelial membrane protein 3 (EMP3) was previously reported to be involved in immunological reactions and in tumor as potential suppressor, as well as a candidate gene for cardiac dystrophic calcification (DCC). Given its assumed role in different disorders, we aimed to generate and perform a generalized phenotypic screening of EMP3-knockout mice.
Materials and Methods: TaconicArtemis used knock-out targeting strategy and generated knockout mice for EMP3. Large-scale phenotyping screens were performed at the German Mouse Clinic (GMC) to obtain a standardized and comprehensive way of phenotyping. The phenotype screens involved tests for nearly 320 parameters in different screening areas such as: dysmorphology, behaviour, neurology, eye, nociception, energy metabolism, clinical chemistry and haematology, immunology, allergy, steroid metabolism, cardiovascular and lung function and pathology. For DCC, screening was performed in our laboratory using the freeze-thaw injury method.
Results: Here, we report on the phenotype of EMP3 knockout. EMP3-KO mice are viable and fertile. Under baseline conditions, an aberrant immunological phenotype was found with nearly 70% penetrance in mutant male mice. Specifically, a lower frequency of T cells and an inverse trend in B cells was observed. Also a higher frequency of B cells was seen in female mutant mice.
For all the remaining screening, no genotype-specific differences were found. Also no calcification deposits were found in the EMP3-KO mice as response to injury.
Conclusion: Large-scale phenotypic screening suggests a role of EMP3 under basal conditions in immunity. Differences in the proportion of various leukocyte subsets from the corresponding wild type were found. Further investigation is ongoing to demonstrate the role of EMP3 as tumor suppressor in a sensitized model. Finally, we excluded the EMP3 gene as candidate gene for DCC on the C57BL/6 genetic background.

EMMA - The European Mouse Mutant Archive

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The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. A full description of these services can be viewed on the EMMA website at http://www.emmanet.org.

The EMMA network is comprised of ten partners who operate as the primary mouse repository in Europe and is funded by NGFN-Plus, institutional funding and the European Commission's FP7 Capacities Specific Programme.

EMMA's primary objectives are to establish and manage a unified repository for maintaining mouse mutations and to make them available to the scientific community. In addition to these core services, the consortium can generate germ-free (axenic) mice for its customers and also hosts courses in cryopreservation.

All applications for archiving and requests for mutant mouse strains are submitted through the EMMA website. Mouse strains submitted for archiving are evaluated by EMMA's external scientific committee. Once approval has been granted depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or spermatozoa cryopreservation. Strains held under the EMMA umbrella can be provided as frozen materials or re-derived and shipped as live mice depending on the customer's needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery. All animals supplied by EMMA are classified as SPF in accordance with the FELASA recommendations.

EMMA is a founding member of FIMRe (International Federation of Mouse Resources) and actively cooperates with other leading repositories like TJL and the MMRRC in the US and BRC RIKEN from Japan.

Fgf9 signalling regulates lens fiber proliferation

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Background and Aim:
Fibroblast growth factor 9 (Fgf9) is a potent mitogen and survival factor required for morphogenesis during embryonic development and numerous biological functions at adulthood. Fgf9 knockout mice exhibit male to female sexual reversal, pulmonary hypoplasia, cardiac dilation, retinal abnormalities, and neonatal lethality (MGI database). We recently established the novel Fgf9Y162C missense mutant Aca12 in a dominant ENU screen. In contrast to the targeted Fgf9 null mutation, Aca12 animals suffer from small eye globes (microphthalmia) and thin lenses (microphakia). In this study, we used Aca12 to get first insights into the role of Fgf9 in primary and secondary lens fiber growth.

Methods:
Prenatal lens core development was histologically investigated at E12.5. Postnatal secondary lens fiber growth of individual lenses was tracked by optical low coherence interferometry (OLCI) at different periods between four and 15 weeks of age.

Results:
We first excluded neonatal lethality and male to female sexual reversal in Aca12 mice. Skeletal investigations at P0 identified a fusion of coronal skull sutures. Moreover, Aca12 exhibited significantly reduced (3.9% - 4.8%; $p<0.01$) lens sizes compared to wild-type controls. Size reduction does not influence transparency and histology of three months old homozygous lenses. However, older lenses developed cataracts. Lens area calculations at E12.5 revealed a significantly reduced (15.3%; $p<0.01$) mean lens core in homozygous animals. In addition, lens thickness enlargement was significantly retarded in the growth periods between P28 and P42 ($p<0.01$), P56 and P77 ($p<0.02$), as well as P77 and P91 ($p<0.04$).

Conclusion:
Our results demonstrate a role of Fgf9 signalling in the regulation of primary and secondary lens fiber proliferation.

Immunophenotyping of complement mutant mice

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The Immunology Screen at the German Mouse Clinic performs multi-color flow cytometry analyses in order to identify immunodeficiencies in mutant mouse lines under baseline conditions. Proportions of main leukocytes subsets and T cells subsets are characterized in peripheral blood. Furthermore, we implemented infection with Listeria monocytogenes as a challenge tool.

We demonstrate how hierarchical clustering analysis applied to the frequencies of leukocyte subsets reveals distinct patterns in wildtype and mutant mice. We show exemplarily the analysis of several complement mutant mice sharing a similar phenotype and wildtype mice, both under baseline and infectious conditions. The complement system is an evolutionary old part of the innate immune system conserved among a wide variety of species. Our analysis illustrates how the complement system influences the shaping of the immune homeostasis and the development of immune response to infection.

Neurological and molecular biological characterization of the mutant mouse line Tom40, the protein that comprises the general import pore of mitochondria

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Mitochondria provide cellular energy by oxidative phosphorylation. Defects of energy metabolism are involved in a variety of human diseases manifesting preferentially in tissues with high aerobic demand such as brain and muscle. Mitochondrial dysfunction is also a key player in neurodegeneration and aging.
The vast majority of mitochondrial proteins is encoded by nuclear genes and then imported into the organelle. By gene trap mutagenesis, we have created knockout mouse models for several genes of the mitochondrial import machinery.
The TOM (translocase of outer mitochondrial membrane) complex mediates the import of all proteins of mitochondria into the intermembrane space and additional the insertion of proteins into the outer membrane. Tom40 comprises the main component of the TOM complex as it forms the general import pore. Homozygous Tom40-/- mice are not viable, embryos die before E9,5. Heterozygous Tom40 +/- mice showed no differences in basic neurological functions like locomotor activity, muscle force and motor coordination. Real-time quantitative PCR revealed a 50%-reduction of Tom40mRNA in heart tissue. In the electrocardiogram (ECG) measurement under anesthesia we detected a decreased P-wave duration and prolonged Q-T and S-T intervals in the mutants indicating conduction impairments.
Our next steps will focus on detailed expression analysis of mRNA and protein level in different tissues. Functional analyses of the respiratory chain activity of heterozygous mice are under progress. Additionally, we will perform embryonic studies like blastocyst preparations in order to find out at which time point Tom40-/- animals are not longer viable.
The analysis of this mouse model of mitochondrial import will lead to a better understanding of this essential pathway in mammals.

Exercise and a stress challenge platform in the German Mouse Clinic

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Work in the Behavioural Screen of the German Mouse Clinic involves studying the consequences of stress and lifestyle on the progression of diseases. Chronic stress has been shown to be a risk factor for the development of neurodegenerative diseases, such as Alzheimer's and Parkinson's Disease, whereas physical activity ("exercise") can be protective. In this context, adult neurogenesis may play a role, since chronic, unpredictable and uncontrollable stress reduces adult neurogenesis, and neurogenesis is reduced in neurodegenerative diseases. Evidence from human studies suggests that exercise mitigates symptoms of stress and anxiety, in addition to increasing neurogenesis in rodents. To study these interactions, we establish acute and chronic stress protocols for use in mice that reliably induce prolonged behavioural changes (i.e. increased anxiety, passive coping), and a wheel-running paradigm to examine the effects of regular voluntary exercise. Restraint is the stressor of choice; this well-defined challenge effectively mimics psychosocial stress in man in terms of phenotype and also decreases neurogenesis in rodents. We will present results of an acute stress protocol indicating that hypo- and hyper-reactive mice can be evaluated by different restraint periods followed by the Open Field Test; a test of anxiety, locomotion and exploration. Results of the wheel-running paradigm presented here reveal the influence of running on the behaviour of mice in the Open Field; and any neurogenetic changes that occur.
Ultimately, we will combine the stress challenge platform with the mouse wheel running paradigm to look at the effects of exercise on stress coping and on disease development in genetic mouse models of neurodegenerative diseases. The marriage of the stress platform with voluntary exercise will provide innumerable possibilities for elucidating the more complex and inducible phenotypes of mutant mouse models that may be extrapolated to the human disease condition.

A new approach in experimental Listeriosis: in vivo imaging of orally infected mice using bioluminescence

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Listeria monocytogenes is a ubiquitous gram positive bacterium and the causative agent of Listeriosis, a food borne disease in humans and animals leading to gastroenteritis, meningitis and sepsis. In pregnant women infection with Listeria may result in spontaneous stillbirth or abortion. The primary site of infection is the intestinal epithelium after ingestion of contaminated food. We generated a bioluminescent Listeria monocytogenes strain carrying an Internalin modification InlAS192NY369S which is able to recognize the murine E-cadherin receptor and thus allowing to introduce the natural (oral) route of infection in mice.
We use this bioluminescent Listeria strain for monitoring the bacterial migration during disease development of orally infected mice in vivo. Using an “in vivo imaging system” equipped with a high resolution CCD camera we can visualize and characterize non-invasively the course of infection in cohorts of mutant and wild-type mice. We present here the first results of different mouse inbred lines showing differences in the intensity of infection as well as time variations regarding bacterial spreading, the crisis of infection and starting of bacterial clearing.
We further give an outlook how we will use this new approach to obtain a detailed insight in the process of crossing the feto-placental barrier during Listeriosis in pregnant mice.

Histopathological phenotyping and characterization of mouse models in the German Mouse Clinic

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Introduction
The phenotypic characterization of genetically engineered mice is an important tool for understanding the genetic basis of human diseases. Using clinical and morphological criteria, the histopathological phenotype is identified bearing in mind inherent phenotypes of the mouse background strain or derived from environmental influences.

Materials and Methods
The 'primary' assessment of mouse models performs a complete morphological analysis of all organs with standard stains. Specific targeted evaluations can additionally be performed, such as radiography, quantitative immunohistochemistry (IHC), electron microscopy (EM) and molecular techniques, which provide further insight into the histopathological findings. Virtual slide microscopy is presently used for archiving of samples and promoting online discussions and conferences worldwide.

Results
From 103 mutant lines analyzed, 43 (42%) showed a pathological phenotype from which 32 were newly identified. We recognized a new mouse model for a human neuropathological inherited disease which is characterized by changes in the peripheral nerve system. Using EM, a reduction in the width of the myelin layer and in the number of myelinated axons was observed. Another mouse model for human 'high turnover' metabolic bone diseases has been determined with lesions rich in osteoclasts and spindle cells which result in abnormal bone remodeling. In addition, a mutant mouse line exhibiting gastrointestinal stroma tumour (GIST) is presently being analyzed with IHC markers.

Conclusion
The histopathological phenotyping of the mutant mice plays therefore a valuable role in a multidisciplinary effort to identify and characterize anatomical changes introduced by the mutation of interest and ultimately establish a relation to human diseases.

Secondary Screen of SIP1+/- mice

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Smad-interacting protein 1 (Sip1) is a transcription factor that plays an important role in neuronal development and is involved in the etiology of Mowat-Wilson Syndrome. A corresponding mouse model that carries a heterozygous Sip1 allele was recently established. A comprehensive phenotypic analysis of these mice was performed in the German Mouse Clinic (GMC) in Munich where the mice displayed reduced pain sensitivity. The following secondary screen was conducted in Bonn and the hypoalgesia was confirmed in von Frey-, Plantar- and tailflick tests. However, no significant changes in nociception were observed in models of neuropathic and inflammatory pain. We performed further electrophysiological and immunohistochemical studies, which focussed on dorsal root ganglions (DRGs) bearing the cell bodies of A-d and c-fibers. Reduced action potential firing and higher persistent sodium currents were found in Sip1+/- capsaicin-positive DRG neurons. Immunhistochemical quantification using specific NaV1.6 antibodies reveals a 30% higher NaV1.6 expression in DRGs of Sip1+/- mice.
These studies suggest an involvement of the TTX-S sodium channel NaV1.6 downstream of Sip1 signalling in thermal and mechanical pain sensation. Further modulation of neuronal development by Sip1, especially in small DRG neurons, may also contribute to altered pain sensation.

Metabolic phenotyping of mouse mutant lines

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Based on the increase of diseases related to disturbed energy homeostasis (e.g. the metabolic syndrome) the identification of genes associated with energy balance is of highest public interest. The German Mouse Clinic (GMC) offers the standardised phenotyping of mouse mutant lines (MML) in 320 variables in 14 different screens. Among those, the Metabolic Module performs a comprehensive analysis of energy balance. It is based on the conflation of different methods for the investigation of disturbances in energy metabolism, body weight regulation and body composition. Following the standard operating procedures developed for the consortium of European Mouse Disease Clinics (EUMODIC) indirect calorimetry is implemented with the aim to detect alterations in metabolic rate in a high throughput primary screen. Thereby, energy expenditure is calculated from gas exchange analysis in an open flow respirometry system allowing the serial measurement of up to 7 mice. Body weight and body temperature are measured; differences in body composition are determined using a non-invasive NMR scanner. Since the start of the EUMODIC based phenotyping one year ago we have successfully detected new metabolic phenotypes: Disturbed body mass regulation was found in 7 of 23 and defective temperature regulation in 4 of 23 analyzed MML. Major changes in metabolic rate were revealed in 6 of 22 MML. We are currently in the process to evaluate the interrelations and correlations of body mass, body temperature regulation, and metabolic rate.

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TMEM132D – a putative cell adhesion molecule involved in panic disorder

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A recent association study identified single nucleotide polymorphisms (SNPs) in TMEM132D which were found to be associated with panic disorder, in particular with the severity of anxiety symptoms in patients affected by panic disorder as well as in patients with unipolar depression. In a mouse model of extremes in trait anxiety, anxiety-related behavior was positively correlated with Tmem132d mRNA expression in the anterior cingulate cortex. TMEM132D may thus be an important new candidate gene for panic disorder and anxiety symptoms. Bioinformatic screening for sites and motifs suggests a role of TMEM132D in cell adhesion but until now its function is poorly understood. The aim of this study is to investigate the function of TMEM132D in detail using in vitro and in vivo approaches. Expression of a TMEM132D-GFP fusion protein in HEK-293 cells confirmed its predicted localization in the cellular membrane. Overexpression significantly increased the number of filopodia-like structures. Additionally, we observed a strong colocalization of TMEM132D with actin filaments as shown by phalloidin staining. Further analyses will involve shRNA studies and a membrane-based Y2H screening which will allow to identify possible interaction partners. The expression pattern of Tmem132d in the brain of wild-type animals at different postnatal stages and in adult mice was analyzed by in situ hybridization (ISH). Conditional knock-out (KO) mice are currently established using embryonic stem cell clones received from the German Genetrap Consortium (GGTC) based on a conditional gene trapping approach. These conditional gene trap clones were injected into blastocysts and chimeric animals were screened for germline transmission by PCR. We obtained two chimeras which transmitted the recombinant allele through the germline. Specific expression of the LacZ reporter gene was analyzed by X-Gal staining confirming the results obtained by ISH. The primary characterization of KO mice is in progress.

Behavioral Changes in G72/G30 transgenic mice

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Genetic studies have identified the G72/G30 locus in psychiatric diseases like schizophrenia, bipolar affective disorders and depression. It represents an evolutionary novel, anthropoid primate-specific gene locus encoding a protein with no recognizable motifs and controversial functions. On one hand it might act as a regulator of the peroxisomal enzyme D-amino-acid-oxidase (DAO), or as a mitochondrial protein promoting its morphology. Here we demonstrate that “humanized” BAC transgenic mice expressing G72 transcripts develop several behaviours related to psychiatric diseases. Proteomic and biochemical analysis revealed G72-induced mitochondrial dysfunctions, with concomitantly increased production of reactive oxygen species. Affected neurons showed deficits in short-term plasticity and capability to sustain synaptic activity. These results identify G72-induced mitochondrial and synaptic defects as a pathomechanism for associated psychiatric diseases.

The role of D-serine in depression-related behaviours

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Glutamate is the most important excitatory neurotransmitter in the mammalian brain. Despite glutamate, the activation of NMDA receptors also requires the binding of a co-agonists D-serine or glycine. D-serine is formed from L-serine by serine racemase (Srr) in protoplasmatic astrocytes. High D-serine levels are found in murine forebrain regions and low levels in the cerebellum and brain stem.
Here we have generated a mouse model with an over-expression of serine racemase under the glia-specific GFAP promoter on a CD1 genetic background. Two transgenic mouse lines showed a significant higher expression of Srr on the transcript and the protein level in many brain regions, with concomitant increased D-serine levels.
Strikingly, the transgenic animals exhibited a decreased propensity towards depression-related behaviours, as shown in the ultrasonic vocalisation test, in the forced swim test (despair behaviour) and in bulbectomized transgenic mice.
In contrast, we did not observe anxiety phenotypes or schizophrenia-related behaviours.
Working memory, as examined in the Y-maze test and operant learning were also not affected by the Srr expression. However, we found that the transgenic mice were more motivated to work for a food reward. We confirmed the depression and motivation phenotype after treatment of CD1 wild type mice with 350 mg/kg D-serine for 5 weeks.
Our results demonstrate that D-serine levels are significantly increased by transgenic over-expression of serine racemase. This increase resulted in a specific behavioural phenotype indicative of a reduced proneness towards depression-related behaviours. Our findings warrant further studies on the possible involvement of D-serine metabolic changes in affective disorders.

Identification of enolase-phosphatase 1 as a potential regulator of anxiety and depression

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In this study, we have begun to map the genes, which are involved in the control of anxiety and depression-related behavior. In the first phase, we tested the behavioral sensitivity of 543 F2 animals from an intercross of C57BL/6J and C3H/HeJ mouse strains in three different models of anxiety and one depression paradigm.
After the behavioral tests we genotyped all animals with 269 microsatellite markers with a mean distance of 5,56 cM. A Quantitative Trait Loci (QTL) analysis was carried out using the program R/QTL and we identified QTLs on several chromosomes (1, 5, 7, 12, 15, 16, 17). Chromosome 5 contained multiple overlapping loci. Interestingly, several studies of anxiety and panic disorders in humans have identified a susceptibility region on chromosome 4, which is syntenic to our QTL region on chromosome 5. Comparison of human and mouse data thus narrowed the candidate region to 14 Mb. A database search provided 160 annotated genes in this area. Enolase-phosphatase 1 (Enoph1) is a strong candidate among these genes. It is strongly expressed in the brain and the parental strains differ in the Enoph1 expression levels in several brain regions. A genetic analysis revealed that the parental strains carry distinct variants of the enolase-phosphatase protein.
Enoph1 is a part of the methionine salvage pathway. A byproduct of this pathway is S-adenosylmethionine, a well-known mood enhancer. We thus assume that the methionine salvage pathway plays an important role in the regulation of anxiety and depression.

Body composition is differentially influenced by exercise and diet in the BFMI mice

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Obesity is the result of interactions between gene variants and the environment. We developed a mouse model for polygenic obesity to investigate the influence that measures of weight reduction like exercise and diet exert on body composition at different stages of life.
The results of our analysis will support the development of a model in human.

Animals of the Berlin Fat Mouse inbreeding line (BFMI) were fed with a standard and high fat diet (9% and 45% energy from fatty acids, respectively) and were kept under different exercise regimes. Animals of the exercise group were provided with a running wheel immediately after weaning. The control group did not have the possibility of additional exercise. We monitored the increase of body weight and fat content between 3 and 20 weeks of development using magnetic resonance imaging (MRI) and the fat distribution at the age of 20 weeks.

Analysis of body weight and corpulence in the four groups revealed that animals of both diets gained less weight and fat if they had the possibility to exercise. Comparing the feeding groups however, showed that diet had a greater influence than exercise, i.e., animals with high fat diet and exercise were still heavier than animals without exercise but with standard diet. Furthermore, total feed intake was not different between the groups. Examining the fat distribution showed that exercise reduced both of the investigated visceral fat depots and the subcutaneous fat tissue, whereas diet had an effect on only one visceral fat depot and the subcutaneous fat tissue.

The presented results make clear that exercise and diet have a different effect on body composition. This needs to be considered when applying measures of weight reduction.

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Influence of chronic exposure to two different high caloric diets on hypothalamic protein abundance in adult C57BL/6 mice

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The unlimited access of humans to high caloric, energy dense foods together with disturbances in the neural control of body weight are major issues concerning the world-wide epidemic of obesity. The hypothalamus is known to be an important part of the brain dealing with energy balance regulation. We studied whether the chronic exposure of mice to high caloric diets leads to changes in hypothalamic protein abundance.

Adult C57BL/6 mice were either fed a high fat (HF), “cafeteria” or control diet. Animals on “cafeteria” diet could choose from three different tasting pellets: “salt”, “biscuit” and “chocolate”. After 18 weeks on diet hypothalami were collected and subjected to proteome analyses. Two approaches for quantification of protein abundances were applied: two-dimensional difference gel electrophoresis (2D-DIGE) and a label-free method using spectral counting.

Mice fed the HF or cafeteria diet gained significantly more body weight and body fat. The effects of the high caloric diets on protein abundance in the hypothalami were rather small. In the 2D-DIGE approach 13 proteins were found to be differentially expressed between the HF and/or cafeteria group compared to controls, 5 of which had a fold change > 1,2. Spectral counting revealed 33 significant changes in protein abundances (fold change > 1,4) between HF and/or cafeteria group compared to controls. One mitochondrial protein that was found upregulated in the HF as well as in the cafeteria group using spectral counting, was also found upregulated in the HF group in 2D-DIGE, being the only identical hit between the two proteomic methods. In both approaches more proteins were found differentially expressed in the cafeteria than in the HF diet compared to the control diet.

Differences in hypothalamic protein abundance in mice exposed to high caloric diets for 18 weeks would likely reflect long term effects or persisting changes of obesity on the brain.

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MUTANOM Mouse Disease Models

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The NGFN project Mutanom aims at the systematic functional characterisation of somatic mutations in breast, prostate and intestinal cancer cells. We complement this systematic approach to cancer cell biology by providing advanced mouse disease models to study selected key oncogenes and oncogene co-operativity in vivo.To this end, we employ Recombinase Mediated Cassette Exchange (RMCE) to integrate mutant versions of oncogenes into the genome in a controlled manner. Importantly, our mouse models will provide inducible and tissue-specific oncogene expression in a pure B6 genetic background. We will study co-operativity of oncogenes in the intestine by crossing oncogene-transgenic mice to B6 APCmin mice, a common mouse model of intestinal cancer. We use culture of primary normal and transformed intestinal crypts as an additional tool to compare the effects of selected oncogenes in colon carcinogenesis.The possibility of systematic in-vivo analysis of the roles of selected mutations in the mouse will likely unravel important aspects of tumor biology that cannot be assessed in cell culture or in retrospective analysis of human tumor samples.

Modeling neuroblastomagenesis from neural crest stem cells in vitro and in vivo

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PURPOSE: Cumulating evidence suggests that neuroblastoma (NB), the most common extracranial tumor of childhood, originates from neural crest stem cells (NCSC). N-Myc has been shown to promote cell cycle progression in NB cells in vitro, and transgenic overexpression of N-Myc induces NB-like tumors in mice. Still, the origin of NB could not be clearly assigned to a defined cell population. To address this question, we established NCSC cells with stable ectopic expression of N-Myc and analyzed their tumorigenicity in vivo.

METHODS: We previously established a multipotent NCSC line, JoMa1, which is kept in an undifferentiated state by conditional expression of c-Myc and which can be differentiated into all derivates of the neural crest. JoMa1 cells were stably transfected with a cDNA encoding N-Myc, and their tumorigenicity was analyzed in immunocompromized nude mice. Tumors were histopathologically evaluated and N-Myc expression was analyzed. Genomic aberrations were analyzed using aCGH. Celllines established from tumors were further analyzed.

RESULTS: JoMa1-N-Myc were capable of growing in cell culture independent of c-Myc expression. Subcutaneous injection of JoMa1-N-Myc into nude mice caused the formation of NB-like tumors. Tumorigenicity of cells was enhanced upon serial transplantation. Tumors originating from JoMA1-N-Myc expressed variable levels of N-Myc. N-Myc expression level was negatively correlated with time to tumor take. aCGH analysis identified a focal amplification of chromosome 8 in tumors with high N-Myc expression levels. Interestingly, treatment of celllines derived from xenografted JoMa1-N-Myc tumors using a Myc-inhibitor inhibited cell proliferation in vitro.

CONCLUSIONS: We show here for the first time that transformed NCSC cells can give rise to neuroblastic tumors, which supports the notion that NCSC are the precursor cells of neuroblastoma. We now aim to characterize the tumorigenicity of JoMa1-N-Myc in a syngeneic mouse model.

A new syngeneic MYCN neuroblastoma mouse model and MYCN-DNA vaccine

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Neuroblastoma (NB) is the most common solid extracranial tumor in childhood. High-level expression of MYCN plays an important role in maintaining the malignant phenotype of NB and is the most significant marker for poor prognosis overall. Recent studies suggested that MYCN is a suitable target for immunotherapy, but up to now, a syngeneic NB mouse model over expressing MYCN is not available to examine immunotherapeutic strategies in vivo.
Here, we report the development of a tetracycline inducible MYCN expressing murine NB cell line syngeneic to A/J mice and a new MYCN-DNA vaccine. The murine NB cell line C1300, syngeneic to A/J mice, was stably transfected with a tetracycline inducible MYCN expression vector system T-REx™ . Stable transfection was verified by real-time PCR and Western-Blot, revealing significant high expression levels of MYCN RNA and protein, respectively. Characterization of the murine MYCN expressing cells in vitro and in vivo will be reported.
Furthermore, a new MYCN-DNA vaccine, based on epitopes encoding for three peptides from the murine MYCN protein sequence with high affinity to the A/J mouse MHC class I allele H2-Kk, was designed and tested in vivo for its ability to induce an antigen-specific immune response. Lymphocytes isolated from A/J mice vaccinated with the MYCN minigen vaccine effectively lysed wild type C1300 cells in cytotoxicity assays in contrast to lymphocytes from control mice. Additionally, lymphocytes from minigene vaccinated mice produced significantly higher amounts of IFN-? after stimulation with irradiated C1300 cells, than lymphocytes from control mice, indicating the induction of cytotoxic T lymphocytes by the vaccine that actively kill even parental C1300 tumor cells with relatively low MYCN expression. We suspect that this effect will be enhanced when the newly established high MYCN-expressing C1300 cells are employed in a similar cytotoxicity assay. Results to support this hypothesis will be shown at the meeting.

Functions of virus-encoded miRNAs during gammaherpesvirus infection using murine gammaherpesvirus 68 (MHV-68) as a model

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The human gammaherpesviruses Epstein-Barr Virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are associated with a number of diseases including tumors and lymphoproliferative disorders. Recently, it was discovered that viruses, like genomes of eukaryotic cells, encode miRNAs. EBV was the first demonstrated to encode miRNAs, and shortly thereafter, other herpesviruses including KSHV and MHV-68 were also found to encode miRNAs. It was proposed that these miRNAs participate in both lytic and latent infection and are involved in virus-host interaction. Since pathogenetic studies of human gammaherpesvirus infections are restricted, animal models are of crucial importance. MHV-68 infection of mice is currently the only small animal model to investigate the role of gammaherpesvirus miRNAs during infection. Up to now, 9 miRNAs encoded by MHV-68 have been described but nothing is known about their function.
In this project, we first want to analyze by 454 sequencing whether MHV-68 might encode more than the already identified 9 miRNAs. Second, we want to investigate their expression patterns in vitro and in vivo by Northern blot and real-time PCR. Third, we attempt to discover potential targets by various approaches. Finally, we plan to analyze the function of the MHV-68 miRNAs in vitro and in vivo by analyzing MHV-68 mutants which lack either all or particular miRNAs.

Analysis of miRNAs in the MCMV system

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Viral miRNAs not only modulate the host environment, but interact with the host miRNA- and siRNA-pathways as well. Since RNAi is an ancient antiviral response pathway, viral miRNAs can either down-regulate the expression of host miRNA/siRNA biogenesis factors, or simply outcompete the cellular small RNAs for effector proteins like those of the argonaute-family.
We therefore chose this system to answer the question of whether virus-derived small RNAs function as competitive inhibitors. To this end, sorting of prominent cellular (let-7 / miR-16) and viral miRNAs (m01-4 / M23-2) into RISC-complexes was investigated was investigated by qPCR. Both monitored viral miRNAs could be detected in Ago1, Ago2 and Ago3 after immunoprecipitation, but showed a preference for loading in Ago2. Levels of cellular miRNAs appeared to be mostly unaffected by MCMV infection.
Deep Sequencing Analysis of MCMV infected cells showed 13% of all detected miRNAs to be of viral origin. All 18 known MCMV miRNAs were present and for MCMV miR-m01-1 an additional miRNA, designated as miR-m01-1-5p, was found. Its presence was confirmed by qPCR.
Interestingly, a 17 nt small RNA was detected in the 5`UTR of IE2.

Host genetics contribute to viral miRNA function in vivo

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MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression at a post-transcriptional level in virtually all eukaryotic organisms and some viruses, particularly herpesviruses. miRNAs are non-immunogenic, stealthy tools for viruses to regulate their as well as host gene expression. The human cytomegalovirus (HCMV) is the major cause of morbidity in immunocompromised patients and allogenic bone-marrow or organ-transplant recipients and the leading cause of congenital birth defects. HCMV miRNAs may provide valuable targets for new urgently needed antiviral drugs. The infection of the mouse with the murine cytomegalovirus (mCMV) is the model of choice to study the biology of CMV infection and to explore the role of CMV genes and of host genes in vivo. Recently, we identified 18 distinct miRNAs encoded by the murine cytomegalovirus. We now performed a two-sided approach to study their function. First, we created knock-out mutants and revertants of the three most abundant miRNAs and performed extensive studies in vivo. Second, we performed RISC pull-downs using monoclonal antibodies to Ago2 to identify their targets. We will report on the biological properties of these mutants and their potential targets.

G-protein coupled receptor 43 (GPR43) is essential for neutrophil recruitment during intestinal inflammation

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Molecular danger signals attract neutrophilic granulocytes (polymorphonuclear leukocytes, PMNs) to sites of infection. The G-protein coupled receptor (GPR) 43 recognizes propionate and butyrate and is abundantly expressed in PMNs. The functional role of GPR43 activation for in vivo orchestration of immune responses is unclear. We examined dextrane sodium sulfate (DSS)-induced chronic intestinal inflammatory response in wild type and Gpr43-/- deficient mice. The severity of colonic inflammation was assessed by clinical signs, cytokine production, colonoscopic and histologic scoring. Chemotaxis of wild type and Gpr43-/- isolated PMNs was assessed through transwell cell chemotactic assay.

In chronic DSS colitis, Gpr43-/- animals showed diminished intestinal migration of PMNs and protection against inflammatory tissue destruction. In contrast to this, no significant difference in PMN-migration and cytokine secretion was detected in a sterile inflammatory model (Air pouch model). These data indicate a pivotal role of the intestinal microflora - being the main source for short chain fatty acids in the Gastrointestinaltract - for recruitment and activation of PMNs. Ex vivo experiments show that GPR43-induced migration is dependent on activation of the protein kinase p38a and that this signal acts in co-operation with the chemotactic cytokine KC. In addition, we demonstrate a genotype dependent significant influence of Gpr43 mediated L-selectin shedding which could explain a PMN-emigration defect while the adhesion of PMNs were obviously not affected. The results indicate a critical role for GPR43-mediated recruitment of PMNs and will help to develop GPR43-directed therapeutic strategies in inflammatory bowel disease.

High-throughput strategies for detection of allergy prone mutant mouse lines using a systemic screening approach

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To identify phenotypic alterations in mice, a systemic allergy screening platform able to detect new phenotypes under baseline and/or challenge conditions with a large number of animals in a short time frame together with a limited amount of biologic sample is required. The aim of this study was to establish such high-throughput technologies for detecting IgE-mediated allergy phenotypes. Total IgE levels in murine plasma are used as first-line allergy screening parameter. Mutant lines showing an interesting phenotype are subjected to a more in-depth assessment using allergen challenge. This includes an allergy model of allergic sensitization and aerosol challenge with the model allergen ovalbumin. Alterations of all immunoglobulin isotypes can be rapidly monitored from a single plasma sample by using a Luminex bead-array technology. Cell fractions (eosinophils, neutrophils, MF, T and B lymphocytes, NK cells) from bronchoalveolar lavage (BAL) are analyzed by one-step, single staining multi-color flow cytometry. A second one-step staining is used for the immune phenotyping of the lymphocyte compartment. Additionally, a single-step quantification of multiple cytokines from BAL fluid is performed. The equivalence and validity of the bead-array technology for the detection of immunoglobulins was verified using established ELISA technology. For the cellular BAL analyses, flow cytometric determination correlated significantly with the differential morphological count using classical cytospins, but consuming less time and manpower. All methods proofed to be highly useful for the high-throughput phenotypic allergy analysis in large cohorts of animals. These high-throughput technologies are likely to provide important advances with regard to pathophysiology, diagnosis, and therapy of allergic diseases .

Dyxin/Lmcd1 - a novel regulator of cardiomyocyte hypertrophy

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Aiming to identify new mediators of cardiac hypertrophy, we applied a genome wide mRNA screen of stretched neonatal rat cardiomyocytes (NRCM). We found the z-disc LIM protein Dyxin/Lmcd1 being upregulated (5.6x, $p<0.001$). Moreover, Dyxin was also induced in several models of myocardial hypertrophy, suggesting a role as a mediator of cardiomyocyte hypertrophy.

To test this hypothesis, we overexpressed Dyxin in NRCM which potently induced hypertrophy (150%, $p<0.001$) and the hypertrophic gene program. Consistent with an induction of calcineurin signaling, the calcineurin-responsive gene Rcan1-4 was found upregulated (3.2x, $p<0.001$). Conversely, knockdown of Dyxin (-75% on protein level) completely blunted the hypertrophic response to stretch and PE. Furthermore, PE-mediated activation of calcineurin signaling was blocked by knockdown of Dyxin as assessed by Rcan1-4 expression as well as an NFAT-responsive luciferase construct (NFATluc). In addition, NFATluc activation by an active mutant of calcineurin A (CnA) could be significantly attenuated.

To confirm these results in vivo, we generated transgenic mice with cardiac overexpression of Dyxin. Despite normal cardiac function, adult transgenic mice displayed significant cardiac hypertrophy (3.9 vs. 3.5 mg/g LV/heart weight, $p<0.05$). This finding was supplemented by a robust induction of the hypertrophic gene program including ANF (3.7-fold, $p=0.01$) and a-skeletal actin (2.8-fold, $p<0.05$). Likewise, Rcan1-4 was found upregulated (+112%, $p<0.05$).

Crossbreeding these animals into CnA-overpressing mice (CnATg) revealed an exaggerated cardiomyocyte hypertrophy (+26%, $p<0.001$) and accelerated development of dilated cardiomyopathy.

In summary, we show that the z-disc protein Dyxin is significantly upregulated in models of cardiac hypertrophy. Dyxin induces cardiomyocyte hypertrophy in vitro and in vivo, while its knockdown abrogates a hypertrophic response. Mechanistically, Dyxin appears to signal through the calcineurin pathway.

Transgenic pigs expressing the mutant insulin C93S for the study of the pancreatic beta cell dysfunction in diabetes mellitus

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We previously established a mutant mouse line showing diabetes which was caused by a point mutation in the insulin 2 (Ins2) gene. The point mutation leads to the amino acid exchange C95S and the loss of the A6-A11 intrachain disulfide bond of the insulin. Here we aimed to establish a transgenic pig model expressing the mutant insulin by additive gene transfer for the subsequent study of beta cell dysfunction in diabetes mellitus. The transgene consisted of German Landrace insulin sequences including 1.3 kb insulin promoter and 1 kb genomic insulin gene sequences with the T to A (C93S) point mutation in exon 3 analogous to the mutant mouse insulin. After successful transfection of fetal fibroblasts of both the German Landrace and the Schwaebisch-Haellisch pig breeds, pooled transgenic fibroblasts were used for the somatic cell nuclear transfer (SCNT). Transfer of reconstructed pig embryos to synchronized recipients resulted in two litters with totally five transgenic offspring. Southern blot analysis showed different transgene signal patterns in the animals. Transgenic pigs revealed unaltered fasting blood glucose levels up to an age of eight months. However, disturbed intravenous glucose tolerance and reduced insulin secretion were detected in one transgenic pig of the first litter at eight month of age. The area under the glucose curve of this transgenic pig was 75% larger, the area under the insulin curve 53% smaller compared to the control. Ongoing analyses comprise glucose tolerance tests in the second litter as well as pathohistologic analysis of the pancreas of both litters. Cells from suitable transgenic founders will be used for re-cloning to establish a new transgenic pig model expressing the mutant insulinC93S for the in-depth study of pancreatic beta cell dysfunction in diabetes mellitus.

MODULATION OF MITOCHONDRIAL FUNCTION AND MORPHOLOGY BY INTERACTION OF OMI/HTRA2 WITH THE FUSION PROTEIN OPA1

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Loss of Omi/HtrA2 function leads to nerve cell loss in different mouse models and has been linked to neurodegeneration in Parkinson's disease and Huntington's disease. Omi/HtrA2 is a nuclear encoded mitochondrial protein involved in stress response and a key mediator of apoptosis. Due to the specific energy needs of the nervous system and the non-dividing character of the implicated cell type, disturbed mitochondrial dynamics are critical for cell survival. The accumulation of dysfunctional mitochondria is characterized by increased production of reactive oxygen species (ROS), decreased mitochondrial membrane potential and damaged mitochondrial DNA. Our study focuses on the characterization of Omi function in Omi knockout mouse embryonic fibroblasts (MEF). This was done with live cell imaging, FACS analyses, electron microscopy (EM) and co-immunoprecipitation with subsequent Western Blot analyses. Loss of Omi/HtrA2 caused an accumulation of ROS and reduced mitochondrial membrane potential. We found increased mitochondrial fusion that correlated with increased levels of the mitochondrial fusion protein OPA1. Co-immunoprecipitation demonstrates direct interaction of Omi/HtrA2 with endogenous OPA1. Complementation with wild-type Omi/HtrA2 protein, but not with a protease dead mutant, rescued the observed increased mitochondrial fusion in knockout cells and restored increased levels of OPA1 to normal. We show for the first time a direct involvement of the protease domain of Omi/HtrA2 in the modulation of mitochondrial dynamics and demonstrate a novel role of this mitochondrial serine protease in the regulation of OPA1 steady state levels. Our results underscore a critical role of impaired mitochondrial dynamics in neurodegenerative disorders.

Treatment of obese Mc4r-W16X knockin mice with suppressors of premature stop mutations

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The melanocortin-4-receptor (MC4R) is a G-protein coupled receptor mainly expressed in the hypothalamus. Activation of MC4R by pro-opiomelanocortin derived peptides like alpha-melanocyte stimulating hormone (MSH) results in promotion of negative energy balance. In humans, mutations in the MC4R gene are the most common cause for monogenic caused obesity. Around 6% of obese people encode mutations in the MC4R.
The mutation W16X is one of 5 known nonsense mutations in the human MC4R gene and is positively associated with obesity. Our in vitro analysis revealed that the MC4R-W16X variant has a loss-of-function phenotype characterized by reduced plasma membrane expression and impaired signaling properties. Incubation of cell cultures with the aminoglycoside G-418 partially restored the mutant phenotype of MC4R-W16X due to the ability of aminoglycosides to mediate a readthrough of premature termination codons while protein biosynthesis.
Furthermore we generated a Mc4r-W16X knockin mouse strain by homologous recombination in embryonic stem cells harboring the stop mutation in the Mc4r gene. Metabolic phenotyping demonstrated that knockin mice are severe obese, hyperphagic and have alterations in hypothalamic gene expression.
Subcutaneous administration of the aminoglycoside gentamicin and the novel stop mutation suppressor Ataluren (PTC124) did not promote weight loss in Mc4r-W16X knockin mice possibly due to the low ability of the drugs to cross the blood-brain barrier. In a next step we aim to infuse the substances centrally into the cerebral ventricles to circumvent the blood-brain-barrier.

EURExpress, a web-based gene expression atlas of the developing mouse embryo

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Genome-wide expression analyses have a crucial role in functional genomics. RNA in situ hybridization (ISH) provides an accurate spatio-temporal description of the distribution of transcripts at cellular resolution. The EU-funded EURExpress consortium has generated a transcriptome-wide acquisition of expression patterns on sagittal sections from E14.5 wild type murine embryos by means of ISH with non-radioactive probes and used this data to establish a web-linked, interactive digital transcriptome atlas. The expression patterns of approximately 18,000 genes were generated, manually curated and textually annotated in a highly informative database freely available to the scientific community (www.eurexpress.org), which contains over 1400 hierarchically organized anatomical terms. The analysis of the data produced so far has determined that about 40% of genes show a specific/restricted pattern of expression. 950 genes, of which 16% have unknown function, showed an exclusive expression patterns in organs such as the central nervous system, ear, eye, skin, liver, skeletal muscle, mesenchyme or salivary glands, representing a unique resource of novel tissue-specific markers. A detailed molecular characterization of the central nervous system revealed, for example, novel molecular regionalization of the thalamus, diencephalons and the telencephalic pallium. Using the annotation of the data we were further able to characterize gene clusters of expression. This genome-wide digital transcriptome atlas is a resource that will help the study of human development and disease allowing to identify tissue specific markers to characterize disease phenotype, to evaluate disease prognosis, to measure therapeutic benefits and to help identifying genes whose mutations lead to disease phenotypes.

Oncogene-mediated tumor metabolome in human breast cancer cells and its role in prognosis

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Overexpression of the receptor tyrosin kinase ERBB2 is involved in approximately 30% of breast carcinomas and associated with poor prognosis. It is known that oncogenic transformation not only affects cell proliferation, survival and apoptosis resistance but also has a profound impact on cellular metabolism. These alterations in metabolism support tumor cell growth requirements and might even contribute to malignancy and ability to metastasize. The aim of our work is to identify metabolic changes that are induced during the course of an ERBB2 oncogenic transformation and to study their prognostic influence in breast cancer. For this purpose we have applied a MCF7 breast cancer cell line with a tetracycline-inducible expression of an oncogenic variant of ERBB2 (NeuT). Affymetrix gene array results show that overexpression of ERBB2 has a great influence on the transcription of numerous enzymes involved in different pathways like carbohydrate, folate and polyamine metabolism, lipid homeostasis, de novo synthesis of nucleotides and redox state. Furthermore the association of RNA expression of several enzymes with metastasis free interval (MFI) in 782 patients with node-negative breast cancer, consisting of three subcohorts (Mainz, Rotterdam and Transbig) has been analyzed. For the most interesting candidates liquid chromatography/mass spectrometry (LC/MS)-based techniques are now being performed to uncover how changes in enzyme transcription affect metabolite levels.

National Genome Research Network

Poster presentation abstracts

Symposium IV

Systems Biology

Data integration architecture for systems biology

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Modern biomedical research is often organized in collaborations sometimes involving dozens of labs worldwide. In particular, in genome research and systems biology complex molecular systems are under investigation that need the generation and interpretation of heterogeneous data for their explanation, for example ranging from gene expression studies and mass spectrometry measurements to experimental techniques for molecular interactions and functional assays and, finally, to biological networks and kinetic models. Extensible markup language (XML) has become the most prominent way for representing and exchanging these data. However, besides the development of standards there is still a fundamental lack of data integration systems that are able to utilize these exchange formats, organize the data in an integrated way and link it with applications for data interpretation and analysis. In this presentation, we present an interactive data integration architecture supporting collaborative systems biology research projects. All components of the architecture are open-source developments, and, thus, can be quickly adopted by researchers. The system is currently in use in three NGFN-Plus and NGFN-Transfer projects. We highlight the specific features of the system in these projects and present already published data in order to illustrate system performance with different use cases.

Molecular Characterisation of Uremic Toxins in silico

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In renal failure, or uremia, the kidney is restrained from filtering and excreting which results in an abnormally high concentration of certain substances in the blood. These retention solutes range from small molecules to large proteins and can cause severe effects such as failure of the cardiovascular system. Recent reviews have identified a list of 114 uremic retention solutes that have been reported in the literature. These uremic toxins were identified in high concentrations in patients with renal failure across multiple studies and have been characterised according to their physico-chemical properties. A further molecular characterisation is, however, still missing.
In order to address this point and in order to allow a prioritisation of these substances on the molecular level we have performed a comprehensive bioinformatics analysis within the IA "New Tools for the Prevention of Cardiovascular Diseases and Disorders in Chronic Kidney Disease". We applied network analysis on a large integrated network of 14 different interaction databases, over-representation analysis with respect to known pathways, gene ontology and other annotation, and statistical classification with respect to physico-chemical properties in order to identify common interaction partners and functional modules that relate these substances. We used support vector machines (SVMs) in order to find discriminating features between uremic retention solutes and other substances taken from the PubChem database. For every retention solute a measure was computed that indicates its similarity to the 'average' uremic retention solute.
In summary, our results deliver the first prioritisation of uremic toxins on the molecular level that will be used for future experimental studies within the project.

A novel large-scale screen to identify modulators of miR-21

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MicroRNAs (miRNAs), endogenous small non-protein-coding RNAs, are primary regulators of differential gene expression in many basic cellular processes including proliferation, differentiation and apoptosis. Alterations in miRNA expression significantly contribute to tumor growth by modulating critical genes. For example, miR-21 is upregulated in breast cancer, prostate cancer, glioblastoma and other cancers. Its differential expression also correlates with tumor stage and disease-free survival.
To identify genes involved in the regulation of miR-21 we have screened an siRNA-library of 4400 selected siRNAs, chosen based on their potential to modulate microRNA biogenesis and include miRNA processing factors, nucleases, transcription factors, kinases, phosphatases and other signalling molecules. For a primary screen, four target sites for miR-21 have been cloned into the 3'-UTR of a luciferase reporter-gene. In this way, changes in miR-21 level after transfection of siRNAs result in changes of luciferase concentration.
We have identified 120 genes that significantly regulate miR-21 activity in this screen. Hits in the primary screen are currently analyzed in further screens. Firstly, changes in miR-21 concentration are validated by using quantitative real time PCR. Moreover, screens are carried out to analyze whether the candidate genes regulate the miR-21 promoter or miRNA maturation, so whether they function at the transcriptional or post-transcriptional level. Genes that have an influence on miR-21 processing will be analyzed for their ability to modulate other miRs to potentially identify general regulators of miRNA biogenesis and stability.
Thus, by these screens we aim to identify general regulator of miR-processing as well as miR-21 specific regulators. The alteration of miR-21 expression by modulating expression of the identified genes might also change tumor development. Therefore, these genes may also serve as therapeutic targets.

Identification and characterization of miRNAs modulating NF-kB signaling pathway

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The Nuclear Factor –kappa B (NF-kB) signaling pathway is involved in a variety of biological processes, including inflammation and cell survival. Deregulation of the NF-kB pathway plays a critical role in many diseases, such as cancer development and progression. The aim of this study is to identify and characterize microRNAs (miRNAs) that regulate the activity of the NF-kB signaling pathway by using a miRNA mimic-library covering roughly 820 human miRNAs. For the miRNA screen a reporter plasmid carrying three consensus NF-kB binding sites followed by a luciferase gene was used. Changes in NF-kB activity after transfection of miRNAs were quantified by assaying luciferase activity. The feasability of this system was proven by knocking down main players of the NF-kB pathway as positive controls. The hits from the screen will be further validated by nuclear translocation assays. Furthermore, the target genes of these miRNAs in the extended NF-kB network will be identified, validated and characterized. Finally, identified miRNAs will be examined in cancer-related assays to determine their oncogenic potential. The outcome of this screen might help to better understand the activation and deregulation of the NF-kB pathway which is of pharmacological importance, mainly because of its role in inflammation and cancer. Moreover, a possible result of this screen might also be the identification of novel drug targets that could be exploited to modulate the NF-kB signaling pathway in disease processes.

Identification of Novel Components in Human P38 MAP Kinase Signaling via Whole-Genome RNAi Screening

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Via stress activation, the P38 signal transduction pathway has impact on numerous biological processes, including cell cycle control, cell death, inflammation and cancer. P38 signaling has been extensively documented to play a role in many chronic inflammatory diseases, cardiovascular dysfunction and other pathologies. To identify novel regulators of human P38 MAPK signaling we performed a genome-wide RNAi screen. We systematically knocked down the expression of more than 21.000 human genes in a P38 specific luciferase reporter cell line and assayed for reporter gene expression reflecting the activation status of P38 signaling. Primary hits were validated by secondary screening using phospho-specific antibodies measuring the changes of the P38 activation level by flow cytometry. By these means, we found enrichment for specific protein families, including kinases, transcription factors, GTPases or G-protein coupled receptors and many so far unknown proteins or proteins that don't have an assigned function so far. In addition, gene ontology analysis showed significantly enriched GO terms such as signal transduction, phosphorylation, cytokine biosynthesis, cell proliferation, cell cycle or cell death among the genes identified in the screen. The top 100 candidate genes identified were further functionally analysed in detail for their involvement in P38-dependent processes enriched in GO term analysis, including cell death, cell cycle progression and cell growth. In addition, 23 of those genes were further classified on the basis of their interaction with known P38 pathway components by Lminescence-based Mammalian Interactome mapping assays. In consequence, we identified a novel upstream regulator of P38 MAPK pathway and characterized the mechanistic basis underlying the ability of this protein to promote activation of this signaling pathway. Thus, our results demonstrate the power of genome-wide RNAi approaches for understanding MAP kinase signaling in mammalian cells.

LifeDB in a new Design: Get new insights from experimental data

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Isolated information originating from small or large-scale data sets is often limited in its use, as different and more comprehensive view are often required to obtain novel and meaningful insights and to draw the relevant conclusions. Only this permits to choose the right candidate genes, proteins and clones for further experiments. Here we present LifeDB, a database which integrates protein localization and, interaction with genomic data from external databases, collaboration partners and data from the IG-Cellular Systems Genomics in Health and Disease. On the one hand LifeDB enables the fast and easy identification of high quality human full length protein coding ORF clones which can be used for localization experiments, functional assays and expression experiments. Clones from the Orfeome collaboration as well as the German cDNA Consortium have been integrated and comprehensively mapped to Ensembl transcripts and various external database accessions. By this approach users of LifeDB are able to search for clones with list of genes which include different gene and protein symbols. On the other hand LifeDB permits to compare protein networks of different cell systems and, hence, to find commonalities as well as the specific differences between these systems. This can be useful to identify new candidate genes, proteins and cell lines for experiments and to test the validity of findings in the light of the cellular context. To this end, interaction and pathway data from several different external databases, e.g. KEGG, have been integrated in LifeDB and have been mapped to each other via gene and protein accessions. Experimental data, e.g. coming from next-generation sequencing, will in the future be used to identify putative missing interactions in cell lines and to link mutations to potential physiological differences characterizing the individual systems. The visualization of networks retrieved from list genes, proteins or clones is possible via Cytoscape which provides a lot of graph algorithms for searching in networks and clustering of network components. Furthermore it allows the visualization of expression data and networks can be downloaded in different formats. LifeDB is implemented in a multi tier J2EE structure which makes it easy extensible and provides an intuitive user web interface.

KEGGgraph: a graph approach to KEGG PATHWAY in R and bioconductor

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The characterization of biological pathways represents a critical aspect of system biology. KEGG PATHWAY, a service of Kyoto Encyclopedia of Genes and Genomes (KEGG), constructs manually curated pathway maps that represent current knowledge on biological networks in graph models. While valuable graph tools have been implemented in R/Bioconductor, there is currently no software package to parse and analyze KEGG pathways with graph theory. We introduce the software package KEGGgraph in R and Bioconductor, an interface between KEGG pathways and graph models as well as a collection of tools for these graphs. Superior to existing approaches, KEGGgraph captures the pathway topology and allows further analysis or dissection of pathway graphs. We demonstrate the use of the package by the case study of analyzing human pancreatic cancer pathway. KEGGgraph is freely available at the Bioconductor web site (http://www.bioconductor.org). KGML files can be downloaded from KEGG FTP site (ftp://ftp.genome.jp/pub/kegg/xml).

RpsiXML - Bridging the R Statistical Environment with the Proteomics Standards Initiative

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The huge and ever-increasing volume of protein-protein interaction (PPI) data poses challenges in many aspects of data management in proteomics. While the introduction of the Proteomics Standards Initiative Molecular Interaction XML Format (PSI-MI XML) eliminated many problems during the data exchange, the community is still missing a powerful computational environment to perform analysis on these data with mathematical, statistical and bioinformatic methods. To address this problem, we introduce RpsiXML, a new open-source Bioconductor package that communicates with the molecular interaction databases supporting the PSI-MI XML2.5 standardization of molecular interactions and provides the programmatic interface between the \R{R} statistical environment with the XML files extracted from the respective databases. We describe the implementation of RpsiXML and how the software package handles different types of data, transforms the data stored in the XML files into graph objects of R, and communicates seamlessly with the statistical methods and tools freely available within R and Bioconductor. RpsiXML promotes the interoperability of PPI data in the computational biology context, and provides tools and interfaces to infer new knowledge from the inspection of PPI data. The package is freely available from the Bioconductor project.

Identi cation of signi cant effects downstream the ERBB receptors in time-resolved protein microarray data

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The analysis of signalling and regulatory pathways in cancer is increasingly moving from single gene or protein analysis to the large-scale analysis of many components. The aim is to gain insight into biological systems on a functional level. In the past a lot of effort was spent on the analysis of DNA and RNA data resulting in information on transcriptional regulation. However, the actual signalling events are mediated on the posttranslational level, such as changes of the phosphorylation state, protein-protein interactions or conformational changes within proteins. Protein array technology can be utilised to measure the protein expression levels as well as the phosphorylation levels of multiple components in parallel, which allows to picture the dynamics of a signalling network directly in a comparable and reproducible way.

The aim of this work was to perform an analysis of activation patterns of proteins in a timecourse in order to find signalling cascades that drive a certain response in various breast cancer cell lines. We propose a statistical method for identifying significant effects in the protein expression profiles over time. A number of generalised additive models using smoothing splines are fitted to the timecourses. Using analysis of variance (ANOVA) significant models are identified and p-values for
time effects are calculated by comparing the most significant curve to a constant model. We show that our method performs well in fitting expected expression profiles after stimulation of the cells, such as the activation of MAPK signalling in response to an EGF stimulus.

With this method for protein microarray data analysis a summary of effects in response to certain stimuli can be found in a quick and convenient manner, and thus presents a useful basis for the detailed analysis of the underlying signalling mechanisms.

Unravelling ERBB-ligand and cell line specific signalling in breast cancer

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Breast tumours are categorized by the expression of several hormone or growth factor receptors. One tumour type is over-expressing ERBB2, an orphan receptor of the epidermal growth factor receptor family. ERBB2 is over expressed in 25-30% of human breast tumours and targeted therapies aiming at its inhibition are already clinically approved. However, only 30% of the patients benefit from this targeted therapy and virtually all patients develop resistance within one year. Our aim is to analyze the crosstalk between different ERBB family members and the signalling cascades downstream of the receptors.
Thus, we employ quantitative protein microarray-based proteomics to study the effect of ERBB ligands in a time-resolved manner. We analyse the three most potent ligands epidermal growth factor, heregulin-ß1, and betacellulin which are specific for distinct receptors. As cellular system we utilize the breast cancer cell lines MCF-7, HCC1954, and BT-474 expressing ERBB1-4 at different levels. The differential activation of major tumour relevant pathways, the MAPK cascade and the signalling through AKT, is quantified using microspot immunoassays to reveal the dynamics of ERBB signalling in different biological settings. Our data show that the activation of downstream signals is both ligand and cell line specific. In a systems biology approach we aim to identify key players responsible for cell line and ligand specific signalling.

Eestrogen-receptor pathway signalling and genomics in mamma carcinoma cancer

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Even though the oestrogen-receptor pathway has been the subject of intensive biochemical and genetic studies we are missing largely the molecular information on tumour development and on dynamic protein interactions. In particular more quantitative approaches are required to gain better understanding of the molecular action of compounds that modify oestrogen pathways and relevant downstream events in mamma cancer on a cellular level. Here, we are applying a tissue culture cell model to elucidate molecular changes of oestrogen receptor (ER) signalling through systematically dissecting the pathway components via RNAi in combination with functional cellular assays. A second goal of this project is the evaluation of chemotherapeutics on this cell model. The primary goals of this project are: (a) to analyse the aberrations of oestrogen receptor pathway in the cell model by mRNA and protein profiling in combination with functional cellular assays and second generation sequencing approaches (b) to evaluate the relevance of anti-cancer therapies through detailed analysis of the response to drug treatment in ER positive and ER negative cell lines (e.g. tamoxifen, parthenolide, rapomycin, gefitinib) on the oestrogen receptor pathway and its signalling components (e.g. mTOR, AKT; PIK3).

Establishing data processing methods to define protein complexes in higher eukaryotes

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The data processing pipeline of DiGtoP is characterized by tagging genes of interest, protein expression, complex copurification and complex analysis performed by mass-spectroscopy methods.
One aim of our project is the development of enhanced methods to normalize and cluster the mass spectroscopic data and to be able to define protein complexes and their components. The results will be integrated in the refinement of the analytical focus in the further experimental rounds.
A new kind of biological background data to be integrated are the short linear motifs (LMs) that play an important role as mediators of protein interactions in a cooperative manner [1]. Many proteins involved in cell regulation pathways (e.g. CBP, P53, Epsin-1) are network interaction "hubs" with interactions established via such short linear motifs in their natively disordered regions. In many cases these short linear motifs also undergo extensive phosphorylation and other post-translational modifications [2].
Such LM-mediated interdependencies may serve as additional edges in the final network to get an higher analytical resolution. Therefore, we want to evaluate if it is possible to get deeper insight in cellular networks by defining the kind of such LMs and by predicting their occurence in protein interactors.

[1] Gibson TJ., Cell regulation: determined to signal discrete cooperation, Trends Biochem Sci. 2009 Oct;34(10):471-82. Epub 2009 Sep 8.
[2] Diella F, Haslam N, Chica C, Budd A, Michael S, Brown NP, Trave G, Gibson TJ, Understanding eukaryotic linear motifs and their role in cell signaling and regulation, Front Biosci. 2008 May 1;13:6580-603.

Integration of paired mRNA and microRNA expression data

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Although sequences of many microRNAs in the human genome have been discovered, their functions and regulation are often unknown. microRNA target databases hold many putative targets for each known microRNA, but they are likely to contain a large number of false positives, due to the very short seed sequence which characterizes a microRNA. In addition, the database algorithms usually do not consider if mRNA and microRNA are located in the same cell compartment at the same time and therefore are able to physically interact. While validating a predicted mRNA-microRNA interaction experimentally is laborious, microarray expression data is more easily obtained for a large number of mRNAs and microRNAs.

We propose to use paired mRNA-microRNA microarray data to get insight into the interplay between microRNAs and mRNAs. For this task, we have used a regularized linear regression approach to model mRNA regulation by microRNAs from microarray expression data. We have chosen the Lasso modification of Least Angle Regression (LARS), which- unlike other regression approaches- performs variable selection and yields a sparse set of microRNAs that together can predict the expression level of a mRNA. While this approach is correlation-based, it has the advantage that it is not restricted to one-to-one associations. One or several microRNAs can serve as predictors of mRNA expression. Within the regression procedure, preference can be given to microRNAs predicted to be regulators of a mRNA by a microRNA target database. Thus, hypotheses about which microRNAs regulate a gene can be created on a genome-wide level. Annotation of genes and microRNAs give insight into their function and regulation within the cell, different tissues or within a disease. The approach is exemplified on the NCI-60 dataset and a cohort of T-ALL patients where both microRNA and mRNA microarray data is available.

ModuleMaster: a new tool to decipher transcriptional regulatory networks

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Summary: Here we present ModuleMaster, a novel application for finding cis-regulatory modules (CRMs) in sets of co-expressed genes. The application comes with a newly developed method which not only considers transcription factor binding information but also multivariate functional relationships between regulators and target genes to improve the detection of CRMs. Given only the results of a microarray and a subsequent clustering experiment, the program includes all necessary data and algorithms to perform every step to find CRMs. This workbench possesses an easy-to-use graphical user interface, together with job processing and command line options, making ModuleMaster a sophisticated program for large-scale batch processing. The detected CRMs can be visualized and evaluated in various ways, i.e., generating GraphML- and R-based whole regulatory network visualizations or generating SBML files for subsequent analytical processing and dynamic modeling.

Availability: ModuleMaster is freely available to academics as a webstart application and for download at http: //www.ra.cs.uni-tuebingen.de/software/ModuleMaster/, including comprehensive documentation. The complete source code is available upon request.

Key words: gene regulation, cis-regulatory modules, regulatory sequence analysis, matrix scan, transcription factors

Identification of target genes related to alcohol addiction and their interaction

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We analyse microarray data obtained from three different rat strains exposed to alcohol by basic statistics and principal component analysis, and determine statistical dependencies between the expression of different genes by Bayesian network inference. The resulting networks of genetic interactions can be divided into a haemoglobin-related, immunoregulatory and a cytoskeletal subnetwork. On the technical side we find synergetic effects in target gene identification when basic statistics (ANOVA) and Bayesian methods are combined.

First Results of Glutamate MRS in Alcoholic Patients during Withdrawal and Continued Abstinence

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Introduction

The purpose of this preliminary evaluation is to determine whether there are significant changes in glutamate concentrations in the anterior cingulate gyrus (ACC) in alcohol dependent patients during early detoxification in comparison to 14 days of abstinence and to healthy controls independent of genetic variances. Whether these changes parallel well described deficits in NAA concentrations was a second goal of this study.

Methods

In a pilot study 19 alcohol dependent patients on the first day of detoxification when the blood alcohol concentration approached 0 as well as 22 healthy controls underwent single voxel MR spectroscopy in a 3T Siemens Magnetom TIM Trio. Spectra were acquired with a PRESS sequence using the following parameters: TE = 80 ms, TR = 3000 ms and 100 averages. Spectra were evaluated with LCModel. The results were scaled with the interpolated water signal at TE=0. We also accounted for the different amount of grey matter (GM), white matter (WM) and CSF in the measured voxel and their different water concentration by image segmentation of a T1-weighted MPRAGE with a in house developed segmentation tool based on the SPM2 algorithm.

Results

The group comparisons corroborate previous results of decreased concentrations of tNAA in the ACC of patients compared to healthy controls. The NAA signal increases within two weeks of abstinence towards normal levels. We also observe a trend for increased Glu in patients on day 1 with a decrease to normal levels on day 14. We further see a correlation between withdrawal symptoms and Glu for day 1.

Conclusions

Alcoholic patients during early detoxification show elevated levels of Glu in the ACC in addition to reduced tNAA. A TE of 80 ms yields good separation of Glu from Gln and less contribution of macromolecules but due to the lower SNR also requires larger voxel sizes and/or longer acquisition times in order to reach sufficient quality for spectral quantification.

Systematic gene expression profiling of a series of mouse models reveals co-expressed genes

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One of the largest expression profiling datasets from diverse mouse mutant lines (MMLs) submitted to the German Mouse Clinic (GMC) were analysed addressing the following questions: 1) How efficient is our strategy of organ selection with regard to the detection of regulated genes? 2) Can we identify groups of co-expressed genes? 3) Is there functional overlap between regulated genes?

Organs for gene expression profiling using cDNA microarray technology were individually selected based either on conspicuous phenotypes in at least one of 14 GMC phenotyping screens or based on previous knowledge of the mutant phenotype. Systematic gene expression analysis of 90 organs from 46 different MMLs identified changes at gene expression levels in 50% of all organs. Several of the regulated genes were repeatedly co-expressed in the same organ of independent MMLs. The gene ontology (GO) annotations did not reveal evidence for functional relation between co-expressed genes. However, among the regulated genes in all organs GO-term analyses revealed over-representation of some functional categories.

With regard to the specific questions that we addressed with our mouse expression profiling dataset we find that: 1) The knowledge-biased molecular phenotyping screen of the GMC is highly efficient in the detection of regulated gene expression in diverse MMLs. 2) We identify groups of genes that are co-expressed in different MMLs. The set of expression profiles that we have generated thus provides a reference library that we may use to relate profiles of new MMLs in the GMC. 3) So far, we find no GO based functional overlap within groups of co-expressed genes (syn-expression groups). Some overlap in GO functional annotations was evident among the entire dataset of regulated genes. Recently, we started to analyse our expression profile dataset for data mining approaches.

Identification and characterization of new clinically applicable target proteins in prostate cancer

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The prostate-specific antigen test (PSA) has been a good marker diagnosis of prostate cancer (PCa). The low specificity limits its use in early detection of PCa. Differential protein expression patterns facilitate to establish a disease-specific protein signature and identify new targets for diagnosis and treatment options. For current study radical prostatectomy tissues were embedded in tissue-tek. 3-5µm sections were prepared and examined by pathologists for confirmed diagnosis. The corresponding regions of cancer and normal on tissue block were sliced out for proteomic application. To increase sensitivity and absolute quantitative proteomic analysis we employed 2D-DIGE. 2-DE followed by MALDI-TOF-MS-MS results revealed distinct protein signature of tumor and normal tissue of individual cancer patients. Important proteins identified included prostatic acid phosphatase precursor (PPAP), a significant overexpressed protein in PCa, tumor suppressors, enzymes involved in glucose and fatty acid metabolism heat shock and cytoskeletal proteins. We performed unsupervised clustering and partition analysis of expression data to find potential candidate can classify normal and tumors. Analyses showed that heterogeneous tumors were selected and more than one protein can classify all samples correctly and need further validation. The expression data is analysed to find correlation between PSA and protein expression. Results showed no correlation of expression pattern with PSA. Interesting proteins were verified for confirmation of proteomic data. Results showed distinct overexpression of DDAH1, Prdx3, Prdx4, EIF4A3, ARGII, FKBP4 and TGM2 in cancer patients where as UCHL1 is downregulated. Since their physiological role in PCa progression is not known functional characterization will be done by altering their expression in LNCaP cells. All stable cell lines were established and further experiments to characterize their role in proliferation, apoptosis and migration are ongoing.

Identification of clinically relevant biomarkers for prostate cancer using a data integration approach

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Introduction

Many potential biomarkers have already been described for prostate cancer, but only a few have made it into clinical practice. In most of these studies, samples have only been analyzed on a single molecular level, omitting important relationships between DNA, RNA and proteins. In this project a data integration approach will be used to improve the biomarker discovery. Screening studies on DNA, RNA and protein level from the same sample will be combined resulting in clinically relevant biomarkers.

Methods and Results

In this project, prostate tissue from 100 patients (50 cancer and 50 controls) were analyzed on DNA, RNA and protein level. On the DNA level, screenings for amplified and deleted regions were performed as well as mutation and epigenetic analysis. On the RNA level, microRNA and mRNA expression levels were analyzed as well as alternative splicing events. Protein levels were analyzed using 2D-DIGE mass spectrometry as well as reverse-phase protein arrays.

The integration of the data sets will be performed by correlation analysis in combination with pathway and meta-data analysis. Deleted or amplified DNA regions will be correlated to mRNA levels. mircoRNA levels will be correlated both to mRNA and to protein expression levels. mRNA expression levels will be matched to protein levels. Additionally, publicly available information about protein-protein interaction, post-translational modifications and pathway knowledge will be used to add more information to the experiments performed.

Conclusions

By integrating the data from the experiments described above, we will be able to find biomarkers with clinical relevance that can be used in further validation studies.

Workflow for integrating gene expression and miRNA data

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Background. Micro RNAs (miRNAs) are small, 21-nucleotide-long non-coding RNAs which regulate approximately 30 % of the protein coding genes in the human organism. Together with AGO proteins, the miRNA forms the miRNA-induced-silencing-complex (miRISC) which interacts with the mRNA based on sequence complementarity. The binding of the RISC to the mRNA leads to either a repression of the translation process or destabilization of the mRNA. The latter comes with a decrease of the mRNA level and thus can be measured with help of gene expression arrays.
Method. A workflow to integrate miRNA and gene expression data based on correlations between the miRNA and the gene expression levels has been developed. By combining the correlations with target predictions we are able to construct a bipartite graph, a graph with connections between miRNAs and genes. Now, connections based on meta information are added, allowing analysis of over-representation of target genes. Together with correlations among genes these connections can be used to complete the bipartite graph. This will enable us to separate direct miRNA-mRNA interactions from secondary effects based on gene-gene interactions. The effectiveness of this workflow is tested with samples of colorectal and prostate cancer.
Conclusion. With this workflow we combine miRNA and gene expression data together with meta information from target prediction and pathway databases. Thereby, a more detailed picture of the regulation mechanisms of miRNAs emerges and may open the possibility to identify affected pathways.

ConsensusPathDB - towards a more complete picture of cell biology

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Functional interactions among genes, proteins, metabolites and other entities in the cell are the key drivers of cellular processes. Knowledge of these interactions will help us understand why such processes develop as they do, and how complex diseases emerge and evolve. Because of their great explanatory power, different methods have been developed to elucidate the networks of physical protein interactions, gene regulatory relations, signaling and metabolism in several organisms. Results from such methods are interspersed in over 300 databases, each of which has its specific focus (protein interactions, or biochemical pathways, or gene regulation, etc.), data format, and bias. In order to obtain a more complete and less biased picture of cellular processes, we have developed ConsensusPathDB – a functional interaction meta-database that integrates heterogeneous interaction networks from human into a single network (http://cpdb.molgen.mpg.de). Our database currently contains over 133,000 distinct functional relations between 32,500 different cellular entities obtained from 13 public databases.

Protein-protein interaction networks have been exploited recently for disease detection and classification and have yielded promising results. Although physical binding between couples of proteins is an important functional aspect of proteins, it is not not the only one. Since the network contained in ConsensusPathDB reveals additional molecular relations, including signaling, metabolism and gene regulation, it can potentially yield even better classification and understanding of complex diseases and may take us a step further towards combating them.

An automated approach for identification of parameter relations resulting in a switch-like behavior of pathway activation

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Computational models of ordinary differential equations (ODE) are widely used to elucidate molecule interactions in complex systems and help developing and testing hypotheses about activation mechanisms of signaling pathways. Initial concentrations are important, but often unknown parameters of ODEs resulting in different system (pathway) response. Distinct responses have been shown to depend on different relative quantities of pathway components. Identification of parameter relations can thus suggest conditions which lead to activation of pathways.

In order to find parameter relations which lead to a switching mechanism for a certain component in a given model, an automated approach to scan the parameter space for initial concentrations of all involved molecule species is combined with a machine learning algorithm. Sampled parameter sets are used for pathway simulation and classified as pathway-activating or non-activating sets. These parameter sets are then subjected to a decision tree algorithm which tests parameter relations and results in splitting rules for dissecting the parameter sets into the classes. As a proof of concept, a model of EGF receptor internalization was analyzed for either a clathrin-dependent (CDE) or clathrin-independent internalization (CIE), yielding component relations previously reported to be responsible for a switch-like behavior of the CIE-pathway (Schmidt-Glenewinkel et al.). In addition, the approach was applied to the Fas-mediated apoptosis pathway predicting important pathway players.

The method can serve as a useful tool in identifying parameter relations evoking a switch-like behavior and thus lead to experimentally provable hypotheses for conditions needed to activate a pathway.

Hierarchical structure, predictive strength and functional annotation of a disease specific protein system in neuroblastoma tissue detected by 33-dimensional toponome imaging microscopy

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Neuroblastoma is a pediatric tumor with heterogeneous clinical behaviour ranging from spontaneous regression to treatment-resistant and fatal progression. Molecular features of neuroblastoma have been studied extensively by ex-vivo transcriptome expression profiling to predict clinical outcome. However, our understanding of the complex protein systems operating in neuroblastoma tissue in situ/in vivo, is still incomplete, due to substantial methodological constraints on the detection of the specific structural features of such systems. Recently, we developed a robot imaging technique capable of measuring hundreds of molecules in one cell and tissue section thereby elucidating what we term the cells `toponome´ (toponome imaging microscopy MELC/TIS) (Ref 1,2,3). We now report on the implementation of MELC to simultaneously measure 33 different molecular components in tissue sections of primary neuroblastoma with contrasting biological behaviour (regression versus progression). The corresponding `power of combinatorial molecular discrimination' (PCMD), used in our study, ranges between 233 and 264combinations per data point. Our analyses reveal 237 and 57 different hierarchically organized protein clusters and four protein cluster motifs in situ at high subcellular resolution, that sharply discriminate between disseminated neuroblastoma tumors with regressive (stage 4S) and progressive behaviour (stage 4), respectively, both at supracellular and subcellular levels of the protein systems´organization. Our data for the first time demonstrate that toponome imaging can be used to predicit neuroblastoma tumor behaviour with high accuracy (100% discrimination) and moreover identify new lead protein candidates for therapeutical targeting in aggressively growing neuroblastoma.

(1) Schubert, W et al Nature Biotechnol. 2006, 24, 1270 – 1278.; (2) Friedenberger, M. et al; Nature Protocols 2007, 2, 2285-2294; (3) Schubert W et al. J Proteome Res. 2009 ; 2009 Jun;8(6):2696-707.

Discovery of cellular modules by high-throughput RNAi screening and automated imaging

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High-throughput RNA interference (RNAi) screenings have become a valuable method for the dissection of diverse biological processes, ranging from cell division in C. elegans to synthetic lethality of cancer cells in man. RNAi libraries that target almost every transcript in the human genome in combination with innovative screening technologies now allow the analysis of increasingly complex cellular phenotypes.
As part of our high-throughput screening platform, we have established a variety of cell-based assays for quantitatively measuring cell survival, cell growth and changes in cell cycle or cell morphology. Multiplexing assays with multiples fluorescent proteins are currently being developed in our laboratory for profiling the activity of multiple proteins interacting in cellular signaling pathways. To systematically identify drug-target interactions and to unravel the mechanisms involved in sensitization and resistance to drug treatment we have established a combinatorial approach of RNAi and compound screening.
For the comprehensive analysis of large scale cell-based screening data, we have developed a set of tools and databases. We have recently launched web cellHTS21 with extended capabilities to analyze single, dual and multi-channel data sets. Furthermore, web cellHTS2 offers custom analysis functions, e.g. plate template configuration or user-defined output formats. The GenomeRNAi database2 hosts data sets of more than 58 published genome-wide RNAi screens performed in Drosophila and human cells and is used by internal and external collaborators.
As part of the NeuroNet, we develop and apply novel assays to assess signaling pathways implicated in neurodegenerative disease (ND). A particular interest are synthetic interactions with mutant alleles of ND proteins, using RNAi and small molecular compounds. We will present our current progress in the NeuroNet consortium.

1http://web-cellhts2.dkfz.de
2http://www.genomernai.org

Hypothesis generation for neurodegenerative diseases pathogenesis by text mining

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Clinical, genetic and biochemical studies show that similar molecular pathways are involved in different neurodegenerative diseases (ND). However, the molecular mechanisms of neurodegeneration are still largely unclear. The Medline database of biomedical literature can be mined to obtain the complete current view on genes, their functions, and diseases. Its size, over 19 M records, and heterogeneity represent a challenge for automated systems.

We have developed several methods for information retrieval and extraction which show significant improvements in accuracy and speed when compared to existing resources. Classifying accurately all the Medline database in a few minutes, and ranking all the human genes in a few seconds allow avoiding important known limitations in text mining, such as restricted vocabularies, and required expert knowledge. Abstract selections by custom naïve Bayesian classifiers for NDs showed high ROC scores: Alzheimer's disease (0.93), Parkinson's disease (0.95), Huntington's disease (0.93), spinocerebellar ataxias (0.93), and amyotrophic lateral sclerosis (0.94). Using 35 thousands abstracts related to Alzheimer's disease, the precision of a probabilistic gene selection is 0.90 when compared to a gold standard. Interestingly, experimental protein-protein interaction data show that this selection contains 32% of genes coding direct protein interactors with the amyloid precursor protein (APP) genetically involved in the disease. Furthermore, the systematic analysis of words in abstracts and associated MeSH terms allows building a comprehensive network of genes, abstracts and phenotypes.

Automatically classified abstracts and genes related to ND may help understanding the pathogenesis of these disorders. New hypotheses can be made on gene sets associated to particular diseases or phenotypes, and contrasted with experimental evidences. Our methods and tools are public and can be applied to any disease or species (http://cbdm.mdc-berlin.de/tools).

Modulation of protein-protein interaction networks by phosphorylation

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Protein-phosphorylation is a post-translational modification common to eukaryotes, regulating protein-protein interaction and conformation. It is especially important in signaling pathways relevant to cell proliferation and disease. We developed a modified version of the yeast two-hybrid system employing active kinases. We use this system to screen for phosphorylation-dependent interactions of proteins implicated in neurodegenerative disease. In a genome wide approach, we have identified more than two hundred novel phosphorylation-dependent protein-protein interactions that show high specificity with respect to human kinases. P-dependent interactions are further analyzed in mammalian cell culture using e.g. immunoprecipitation strategies. The resulting network reveals highly connected modules that link proteins involved in neurodegenerative disorders to well-studied signaling pathways. It also provides a framework for the modeling of dynamic cellular interaction patterns.

An interaction network links proteins of the ubiquitin proteasome system to neurodegenerative disease processes

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The accumulation of misfolded proteins in insoluble protein aggregates is a common feature of neurodegenerative disorders such as Alzheimer's, Parkinson's or Huntington's disease. Currently, the molecular mechanisms that lead to protein misfolding and neurodegeneration are not well understood. The ubiquitin proteasome system (UPS) consisting of ?1500 proteins is critical for the degradation of many folded as well as misfolded human proteins. Also, perturbations in this system have been implicated in the pathogenesis of various neurodegenerative diseases. However, detailed knowledge about the protein complexes that degrade neurodegenerative disease proteins such as huntingtin, ?-synuclein, tau or SOD1 is still missing. Based on literature information, we have generated a list of ?150 human proteins that either cause neurodegeneration or are associated with neurodegenerative disease processes. From this list, 128 full length ORFs were sub-cloned into the yeast two-hybrid bait vector pBTM116 and screened against a selected library of 987 human UPS proteins using an automated yeast two-hybrid system. A large network comprising 988 high-quality yeast two-hybrid interactions was created, which links proteins involved in neurodegenerative disease processes such as ?-synuclein or huntingtin to key proteins of the UPS system. These interactions are currently validated using alternative methods in mammalian cells such as FRET or LUMIER. Furthermore, cell-based assays are established to investigate the effects of identified UPS proteins on the ubiquitination of neurodegenerative disease proteins. The interaction data may help us to identify novel protein complexes that are critical for the degradation of neurodegenerative disease proteins, which might be targets for future therapy development.

Constructing a functional network for Huntington's disease by quantitative proteomics and probabilistic modeling

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Huntington's disease (HD) is a neurodegenerative disorder caused by the expansion of CAG repeats in the huntingtin gene. The corresponding huntingtin protein with extended polyQ tracts accumulates in neurons and causes cell death via incompletely understood mechanisms. Here, we used mass spectrometry-based quantitative proteomics to measure changes in cellular levels of more than 6,000 proteins upon overexpression of inducible Huntingtin constructs in a neuronal cell model. We combined our proteomic datasets with probabilistic genome-wide network models to construct a functional network specific for HD. Our network reflects several known disease processes such as oxidative stress response and altered neuronal morphogenesis and allows us to infer new disease mechanisms. Collectively, our data indicates disease-specific networks generated by quantitative proteomics and probabilistic modeling are a valuable resource and can help to understand disease pathogenesis.

Analysis of non-coding RNA function and evolution

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Using methods that we developed for using the expressed sequence tag databases for 3'UTR gene end prediction, we are able now to find traces of transcripts expressed in low amounts and sometimes of small size. These are often ignored as they are small and tend to be non-coding but their presence in the databases is unmistakeable. We are collecting evidence of transcripts expressed from pseudogenes that are natural antisense transcripts of the parental genes. We are also studying the relation between antisense transcription and the alternative splice of the sense gene. Our objective is two-fold. On the one hand, we pursue to answer basic questions about the function and evolution of non-coding RNAs (how do large antisense RNAs evolve? how do they control gene splicing and expression?). On the other hand, we will try to link non-coding RNAs and splice variants to ageing, to find mechanisms involved in diseases that affect mostly the elderly such as cardiovascular disease, neurodegenerative disease and cancer.

Cellular miRNAs with inhibitory or promoting effect on Varicella Zoster Virus growth

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MicroRNAs (miRNAs) are approx. 22nt long, non-coding RNA molecules which regulate expression of target genes mainly on the posttranscriptional level. A wide range of organisms including plants, mammals and viruses has been found to encode miRNAs. These small molecules play important roles in many cellular processes such as development, differentiation, apoptosis and tumorigenesis. Moreover, cellular miRNAs are thought to represent a defence mechanism against pathogens while viruses, e.g. herpesviruses, employ miRNAs to modulate the host cell in support of viral replication or persistence. Herpesviruses are large DNA viruses which establish life-long latency in the host. This burdens the host with a risk for future reactivation and disease. A member of the alphaherpesvirus subfamily, Varicella Zoster Virus (VZV), causes chickenpox and – upon reactivation – gives rise to shingles. Surprisingly, in contrast to all other herpesviruses investigated no VZV-derived miRNA could be identified so far, suggesting that VZV either uses cellular miRNAs for its own replication or that there is a counteractive miRNA-based cellular immune defence mechanism which is inhibited by VZV (thus it does not make sense for VZV to express miRNA itself).

In order to test these hypotheses, we performed a screen using approx. 420 synthetic human miRNA mimic and inhibitor molecules to identify miRNAs with inhibitory or promoting effect on virus growth. One proviral and 18 antiviral candidates showed significant effects with both mimic and inhibitor. Nine candidates were analysed further. Here, we present the validation results of these candidates.

Monte Carlo strategy to develop a systems biology of cancer

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Cancer is known to be a complex disease and its therapy has turned out to be difficult. Much information is available on molecules and pathways involved in cancer onset and progression. By processing literature information and pathway databases of twenty different signalling pathways known to be relevant for cancer (like Wnt, Notch, BMP, Fas, Trail, EGF, IGF, Hedgehog, etc.), we have developed a large mathematical model of these pathways comprising more than 800 individual components using the PyBioS modelling and simulation platform (http://pybios.molgen.mpg.de). Although the development of large detailed mathematical models is difficult, the benefit one could gain using their predictive power is tremendous. The development of such detailed mathematical models is not only hampered by a limited knowledge about the topology of the cellular reaction network, but also by a highly restricted availability of detailed mathematical descriptions of the individual reaction kinetics along with their respective kinetic parameters. To overcome this bottleneck we introduce an approach, based on a Monte Carlo strategy, in which the kinetic parameters are sampled from appropriate probability distributions and used for multiple simulations in parallel. Results from different forms of the model (e.g.
a model that resembles a certain mutation or the treatment by a drug) can be compared with the unperturbed control and used for the prediction of the effect of the perturbation. Using the large cancer model we demonstrate the value of the strategy by generating predictions of the effects of potential drug targets, such as AKT, PI3K, Raf, IRAK, MDM2, MEK, on the other model components and the state of the cell (proliferation, cell arrest or apoptosis). This approach makes it possible to study the effects of drugs or mutations of oncogenes or tumor suppressor genes on the cellular interaction network.

IG Mutanom - Systems Biology of Genetic Diseases

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Cancer, like many other diseases, is caused by disturbances in the complex networks of biological processes in the organisms. Prevention, diagnosis and therapy of these diseases require a detailed understanding of these processes in health and disease. Application of techniques from the area of functional genomics on the individual patient, combined with the development of systems, that are able to model the disease process are now required. The Mutanom project (WWW.MUTANOM.ORG) is an Integrated Genome Research Network (IG) funded through the NGFN Plus Research initiative. The IG Mutanom aims to characterise the functional consequences of somatic mutations and to develop Systems Biology models that predict the outcome of such genetic alterations on a molecular pathway level, cellular and organism level. Initially our effort will concentrate on characterising „driver“ mutations i.e. mutations that occur in cancer due to selective pressure promoting cancer progression. A core set of mutations that frequently occurs in breast, prostate and gastrointestinal cancer tissues has been identified (COSMIC) and additional mutations will be selected through new generation sequencing approaches. A predictive model will be developed from the quantitative molecular information on signalling pathways obtained from combining functional genomics, proteomics, cellular assays, model organism and clinical data. The developed model and pathway information can then be applied to other genetic diseases and will be systematically exploited to identify new drug targets and improve our understanding on the action and side effects of drugs. Hence, we expect this approach and the combined infrastructure to become a key instrument in improving diagnosis and therapy of cancer and many other complex diseases. The aims and overall structure of the project will be reported here.

Serum profile as biological system to identify deregulated genes in human cancer

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Seroreactivity patterns defined as complex human autoantibody reactions against numerous tumor-expressed antigens are investigated for their potential as minimal-invasive, biological marker for several cancers. We provided first evidence that seroreactivity patterns are able to differentiate glioma patients not only from healthy controls, but also from patients with other neoplastic and non-neoplastic brain pathologies with high sensitivity and specificity. To further increase the number of potential antigens we screened an expression library of about 38 000 E.coli expressed clones with 150 sera of patients with various neoplastic and non-neoplastic diseases. We compiled a protein macroarray of the resulting 1827 immunogenic clones, 509 of which represent inframe human proteins. We used this protein macroarray to establish a complex seroreactivity pattern for glioma patients by screening of 57 glioma and 60 healthy sera for autoantibodies against the 1827 immunogenic clones. Using a linear Support Vector Machine approach we were able to differentiate glioma sera from sera of the healthy controls with a specificity of 90.45 %, a sensitivity of 85.93 % and an accuracy of 88.49 %. Altogether we found 46 immunogenic clones including 16 inframe clones that were highly informative for this differentiation as determined by their AUC value. We present evidence for the applicability of serum profiling for systematic detection of biological markers in glioma patients. In the future, serum profiling may also prove useful for a personalized, immune based therapy by providing putative targets.

Integrating univariate and multivariate statistics with pathway database information for the analysis of microarrays

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DKFZ

A strategy will be outlined that integrates univariate and multivariate testing methods for microarrays with pathway database information. When multivariate testing is done, the gene groups to be tested are usually selected prior to the analysis, either based on pathways that are assumed to be affected or more general characteristics such as kinase activity. Because of this static nature, this approach is unable to go beyond prior assumptions. Univariate testing is usually done without any prior selection but has the drawback that it results in a list of differentially expressed genes that is often extensive and difficult to interpret biologically. We combined the benefits of both approaches, the results from univariate testing are used to select gene groups for multivariate testing based on gene interactions. The Rank Product was used for univariate testing and the Globaltest for multivariate testing. Gene interaction information was obtained for the BioCarta database freely available from the NCI website.The results of this method are easy to interpret in a biological context and are founded statistically.

GeNGe - Systematic Generation of Gene Regulatory Networks

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Reverse engineering algorithms are valuable tools for inferring gene regulatory networks from gene expression profiles. Validation of performances is a key aspect for showing the benefit of such algorithms. This can be done under a controlled framework starting with artificial data. We developed a web application called Gene Network Generator (GeNGe) for automatic generation of small as well as large scale gene regulatory models. The constructed networks are composed of an mRNA and protein layer. Different types of networks can be generated, such as random networks, scale free networks, networks assembled of small network motifs, pre-defined networks, and user-defined networks. A nonlinear differential equation system of different kinetics with adjustable parameters is constructed and numerically solved to perform global or local perturbation experiments in silico. Eventually, all simulation results (as flat files) and the gene regulatory network model (as SBML) are downloadable. GeNGe can also we used for network topology studies. Here we show an analysis of a network by a set of single knock-down experiments of different degrees. This shows that the knock-down effect decreases strongly with the network distance (shortest path length) from the silenced gene and vanishes nearly after a length of three.

MoGLI – systems biology analysis and modeling of Hedgehog/GLI signaling and connected regulatory networks implicated in cancer

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The Hegdgehog (Hh) signaling pathway plays a pivotal role in many different biological processes. During embryogenesis it is implicated in pattern formation, differentiation, proliferation, and organogenesis. During adult live stages the pathway is needed for stem cell proliferation and tissue repair. A malfunction of the pathway caused by persistent activation or inappropriate reactivation often leads to cellular hyperproliferation and contributes to different malignant diseases such as basal cell carcinoma, lung, prostate and pancreatic cancer. Therefore, it is of considerable interest to get a precise understanding of the exact molecular mechanisms which control the different functions of Hh/GLI signaling.
The expression of specific target genes underlying the regulation of Hh signaling is controlled by GLI transcription factors, which are the downstream effectors of the pathway. The aim of the bi-national project MoGLI founded by BMBF and Gen-AU is to undeceive the intracellular signaling events of Hh/GLI and the interaction with other regulatory networks leading to cancer. An important assumption for the understanding of complex biological processes is the availability of a sound set of quantitative and time-resolved measurements. An ideal and efficient tool for the fast and reliable profiling of cancer associated changes of the proteome is the protein microarray platform. This technique allows robust analysis of total or posttranslationally modified proteins from small sample amounts. Together with our cooperation partners we will integrate the obtained data into a computational model of Hh signaling. This will allow us to make predictions how a cell reacts under specific conditions. The results will be verified by further biological experiments. Finally, the interplay between biological experimentation and computational analysis will be used to improve the search for valid drug targets which may help to overcome cancer induced by dysregulated Hh/GLI signaling.

Gene List Significance Index (GLSI) improves our method High Performance Chip Data Analysis (HPCDA) dramatically

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After successfully publications of our method High Performance Chip Data Analysis for several datasets (e.g. 1-3), validating and comparing it to SAM and dChip in (4) and transferring parts of this tool for analyzing red/green miRNA chips and FACS data (5) we want to present here an important improvement of HPCDA.
For utilization of tools, downstream of chip data analysis (e.g. Ingenuity, DEEP, or DAVID, but also systems biology), it becomes more and more relevant to obtain gene lists with high accuracy. There is no method for quantifying the quality of gene lists (GLs) available today. We are trying to change this with the new Gene List Significance Index (GLSI). It is a relative value and makes GL rankings independent of normalization methods. A randomly selected GL w/o significant genes achieves values near 1.0; below 1 are GLs of normalization or control genes, not at all significant. With increasing fractions of true positive or high FC genes, GLSI also increases. Only with a quantifier for GL quality you can objectively rank a list of extracted significant genes in a decreasing order of significance. We here can show with GLSI, that neither FC nor t-test, Change calls, nor are other values sufficient to rank genes in an optimal way. Our new HPCDA-Score achieves much better rankings. With Gene List Significance Index we could improve our expression profiling method High Performance Chip Data Analysis. With the individual HPCDA-Score we could rank all selected HPCDA-significant genes in decreasing order of significance. We than calculated the GLSI for all GLs of 10 up to all genes and selected the GL with maximum of Gene List Significance Index. So it is sure that every additionally selected genes reduces the significance of the selected GL.

References:
1. Röck J, Schneider E, Grün JR, et al. Eur J Immunol 2007; 37: 3564–3575
2. Biesen R, Demir C, Barkhudarova F, Grün JR, et al. Arthritis Rheum 2008; 58/4: 1136-1145
3. Tokoyoda K, Zehentmeier S, Hegazy AN, Albrecht I, Grün JR, et al. Immunity 2009 May;30(5):721-30
4. Menßen A, Edinger G, Grün JR, et al. BMC Genetics 2009
5. Steinbrich-Zöllner M, Grün JR, et al. Cytometry, Part A 2008; 73A: 333–340

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Dynamic modeling of biochemical reaction networks with SBMLsqueezer 1.3

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Dynamic modeling of biochemical reaction networks is a demanding and highly error-prone task. Here we introduce a five-step modeling pipeline that ultimately leads to a mathematical description of a biochemical reaction system. We discuss how to automate each individual step and how to put these steps together: First, we create a topology of interconversion processes and mutual influences between reactive species. The Systems Biology Markup Language (SBML) encodes the model in a computer-readable form and allows us to add semantic information to each component of the model. Second, from such an annotated network, the procedure known as SBMLsqueezer generates kinetic equations in a context-sensitive manner. The resulting model can then be combined with already existing models. Third, we estimate the values of all newly introduced parameters in each created rate law. This procedure requires that a time series of quantitative measurements of the reactive species within this system be available, because we calibrate the parameters with the aim that the model will fit these experimental data. Fourth, an experimental validation of the resulting model is advisable. Fifth, a model report is generated automatically to document the model with all of its components. For a better understanding, we will begin with an introduction to current standardization attempts in systems biology and generalized approaches for common rate equations before discussing computer-aided modeling, parameter estimation, and automatic report generation. Finally, we discuss possible further improvements to our modeling pipeline.

National Genome Research Network

Poster presentation abstracts

Symposium V

New Technologies

Analysis of biomarkers in FFPE tissues – What about uPA and PAI-1?

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Background
Node-negative breast cancer patients bearing only low levels of Urokinase-Typ Plasminogen Activator (uPA) and its inhibitor PAI-1 (Plasminogen Aktivator Inhibitor-1) have a very low risk of recurrence; thus, these patients may be spared adjuvant chemotherapy. Currently, both proteins are detected and quantified by an enzyme-linked immunosorbent (ELISA) assay using fresh or frozen tissues that are rarely available in most hospitals. The ultimate aim of our study is to establish a method for quantitative analysis of both markers in formalin-fixed, paraffin embedded (FFPE) tissues which are the major source of clinical tissues in hospitals worldwide.

Methods
Antibodies specific for uPA and PAI-1 were validated using Western blot. We adapted our recently established methodology for protein extraction from FFPE tissues for optimal isolation of uPA and PAI-1. A pilot study for quantitative measurements using protein extracts from FFPE breast cancer tissues and protein microarray technology was performed.

Results
We found three anti-uPA and two anti-PAI-1 antibodies that showed specific signals in Western blots. We successfully established a protocol for isolation of uPA and PAI-1 from FFPE breast cancer samples. For protein microarray-based quantitative analysis two anti-uPA antibodies and one anti-PAI-1 antibody were found to be specific and sensitive enough to detect expression differences in patient samples.

Conclusions
The results of our study fortify the use of FFPE breast cancer tissues for quantitative analysis of uPA and PAI-1 in routine clinical settings. With our tools developed here the next steps are: to compare the results from FFPE tissues with measurements from frozen tissues, to start a comparative analysis of uPA and PAI-1 in a larger series of FFPE tissues, and to compare protein microarray with ELISA data in order to define standards for the analysis of uPA and PAI-1 in many hospitals.

Mapping signaling networks in formalin fixed and paraffin embedded breast cancer tissues

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Background: In most hospitals world-wide clinical tissues are typically formalin-fixed and paraffin-embedded (FFPE). New approaches and developments in targeted cancer therapy have raised the need to establish novel tools for precise protein quantification in FFPE tissues. New techniques being able to detect the entire spectrum of deregulated pathways in tumors before, during, and after treatment are required to assess success or failure of targeted therapies and to conceive why only a subset of patients responds to individualized treatments. The aim of our study was to establish and optimize methods for relative and absolute protein quantification in FFPE tissues with special emphasis on HER mediated pathways in breast cancer.
Methods: Using a recently developed technology for extraction of full-length proteins from FFPE tissues, we evaluated >50 commercial antibodies for specificity in lysates from FFPE breast cancer samples in Western blots and reverse phase protein arrays (RPPA). Purified HER receptor proteins were used for measuring absolute protein concentrations in FFPE tissue extracts.
Results: We confirmed specificity of 35 commercially available phosphospecific and non-phosphospecific antibodies using Western blots with protein extracts from cell lines and tissue extracts from breast cancer patients. Spiking known amounts of purified HER receptor proteins in HER receptor negative tissue extracts allowed us to precisely measure abundances of HER-receptors in FFPE breast cancer tissues using RPPA technology. Adequate controls were designed.
Conclusions: Although the role of proteins as important markers for many diseases is increasing, their robust quantitative analysis is still not possible in routine clinical settings. Hence, the results of our project will serve as a basis for the development of in vitro diagnostic techniques for the quantitative measurement of deregulated HER receptors and downstream signaling proteins in most hospitals.

iCHIP, the data management platform for NGFN-plus projects

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Our in-house iCHIP database (http://www.ichip.de) was originally developed to operate as a gene expression database. Given the rapid development of new technologies in molecular biology, the amount and heterogeneity of available data has increased dramatically. The functionality of iCHIP has therefore been extended to include proteomics, matrix-CGH and microscopy images. A comprehensive user and project management is implemented, which allows user-dependent rights for reading and writing of specific data and several application areas. Furthermore, standardized exchange formats have been included and standardized interfaces for communication with third party products implemented.

iCHIP has been funded mainly by NGFN, starting in the period of NGFN-I and followed by NGFN-II as well as NGFN-plus. Several NGFN-networks have used iCHIP as their database backbone during the three periods of funding. A wide range of experimental data sets generated by different laboratories in the area of transcriptomics, genomics and proteomics have been stored and are currently maintained in iCHIP. Beside NGFN, It serves as the data backbone for different German and European consortia in the fields of translational research, clinical cancer research and systems biology. Within NGFN-plus three consortia (IG Brain tumor network, IG Neuroblastoma Genome Interaction Network and IG Genetics of Alcohol Addiction) are contributing to the standing of iCHIP as database of translational research.

Mutation detection in heterogeneous cancer samples using SNPstream:

A feasibility study

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The detection of mutations in tumor samples is an important task in order to elucidate the origin and progression status of cancer cells. This information is also essential for deciding about the best option for therapeutic intervention. A recent approach applied for high-throughput somatic mutation analysis in tumors is the utilization of genotyping platforms, such as Sequenom, that have originally been designed to detect SNPs (see e.g. Thomas et al. 2007). A prerequisite is the ability of the method to not only measure reliably heterozygous genotype calls but to quantitatively measure allele frequencies down to the low percentage range. In this study, we evaluated the SNPstream genotyping platform from Beckman Coulter for this task. Specifically, we conducted two experiments using a panel of good performing SNPs and simulated mutations of varying allele frequency by pooling DNA from different individuals in different concentrations. From the known genotypes of the individuals and the pooling scheme, it is easy to compute expected allele frequencies for the different SNPs in the produced pools. The comparison between the expected allele frequencies and the allele frequencies measured on the SNPstream platform shows high correlation between the two which means that mutation detection in cancer samples is generally possible with this approach. In a second pooling experiment, the lower border (very low frequency of the mutation) was assessed in particular. Altogether, the results are encouraging that SNPstream is a suitable platform for detection of mutations of varying allele frequency.

References

Roman T. Thomas et al., High Throughput Oncogene Mutation Profiling in Human Cancers, Nature Genetics Vol. 39 No. 3, 2007

Time Lapse Analyzer (TLA): A freely available application for high throughput processing of time-lapse microscopy image data

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Modern microscopes enable the highly parallelized recording of thousands of images in high-throughput experiments. Here, especially the use of time lapse microscopy – where recordings of the same image regions are repeatedly taken in regular intervals over an extended period of time – can provide new insights into the migratory and mitotic potential of cells. Possible applications for this new field of imaging are manifold and reach from cell counting and wound healing assay analysis to measuring the movement of entire cell populations.

To exploit the high potential of time-lapse microscopy and to automatically explore the accumulating image data, we developed the Time Lapse Analyzer (TLA) - a freely available, cross-platform, open source MatLab application.

The TLA provides various image processing and segmentation functions which can interchangeably be combined and adjusted in a stack-like fashion to accomodate individual applications. The software can be extended by self designed MatLab image processing functions via an easy to use interface. The TLA is especially suited for multi-target object tracking, though it also provides tools for wound healing assay evaluation and mitosis analysis as well as video processing and image enhancement functions. The software download for the TLA, the source code, some example applications as well as a detailed program documentation can be found on the project web page: http://www.informatik.uni-ulm.de/ni/staff/HKestler/tla/

DETECTION OF TUMOR-SPECIFIC SOMATIC MUTATIONS BY WHOLE TRANSCRIPTOME SEQUENCING OF A CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA

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Approximately half of acute myeloid leukemia (AML) patients have at least one chromosomal aberration, whereas the other half classifies as cytogenetically normal (CN-AML). Most of the genetic events that initiate the disease are still undiscovered.
To identify tumor-specific somatic coding mutations, we sequenced the transcriptome of a CN-AML and a matched remission sample by second-generation sequencing technology (Illumina GAII). SNPs were called with the MAQ software. Additional filters were applied to exclude known and possible sequencing artifacts.
In a single GAII run we generated 50.4 and 15.6 million 36 bp paired-end reads of the CN-AML and remission sample, respectively, which mapped to exons of UCSC genes. Only 6.5% of reads for the AML and 5.0% reads of the remission sample mapped to intergenic regions. To determine our detection rate we used a non-redundant gene set based on the Ensembl gene annotations as reference. This set contained 35,568 genes, of which we sequenced 10,896 with an average coverage of 7 or greater. 6,087 genes were sequenced to an average coverage of 20 or greater. By comparing the 63,159 SNPs discovered in the CN-AML sample with the respective results in the remission sample, we identified 3 non-synonymous mutations exclusive to the tumor sample. The mutations were confirmed on the genomic level by capillary sequencing. Among them is a nonsense mutation affecting the RUNX1 gene, which forms a well known fusion gene in AML and a missense mutation in the tumor-associated gene TLE4 which interacts with RUNX1. A second missense mutation was identified in the SHKBP1 gene which acts downstream of the AML-associated FLT3 regulatory pathway. Two additional mutations were false positives.
These results demonstrate that our technique of whole transcriptome sequencing is an efficient method to discover novel mutations in AML.

The Heidelberg-Tübinger-MRNET experience: Molecular karyotyping of 175 parent-patient-trios with unexplained mental retardation by Mapping 6.0 SNP arrays

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Only few autosomal mental retardation (MR) genes have been identified to date and none seems to be a common cause of MR. The primary goal of the MRNET-project is to identify such genes using different technological approaches. Recent developments in genomic microarray technology (array-CGH / molecular karyotyping techniques) allow genome wide detection of submicroscopic chromosomal alterations. Among clinically unselected MR-patients with a normal karyotype, 10-15% carry submicroscopic de novo deletions and duplications that are not seen in healthy controls and are likely to be for the cause of their MR. In contrast, the majority of benign copy number variations (CNVs) are inherited. The goal of the MRNET study is to find and define small submicroscopic deletions/duplications in clinically well defined patients with MR for which other causes of their disorder have been excluded. Using this approach we expect to find novel genes which are candidates to be tested in a large cohort of MR patients.
In this study we took advantage of a so-called trio-analysis approach (father, mother, affected child with unexplained MR) to drastically reduce elaborative, costly and time consuming experiments of yet undescribed CNVs using Mapping 6.0 SNP arrays. In a cohort of 175 patients a total of 5292 CNVs (mean: 30,24 CNVs/patient) had been found. Out of these, 346 CNVs (mean: 1,97 CNV/patient) have not been reported previously as common benign variants or where only partially overlapping with known CNVs. However, the number of de novo and thus potentially pathogenic CNVs was reduced to 29 by synchronous trio analysis. In conclusion, analysis of parental DNA was shown to be inevitable for the validation of CNVs in the majority of cases justifying a priori trio-analysis. Compared to array-CGH, trio analysis in SNP-arrays has furthermore the potential to unmask the parental origin of CNVs as well as the detection of Uniparental Disomies in a single experiment.

RPPanalyzer: an R-package to link experimental RPPA data with Bioinformatics

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Reverse phase protein arrays (RPPA) emerged as a method to screen large sample sets. However, the routine RPPA application required the development of suitable software tools for data analysis and data presentation along with the development of benchwork protocols equally useful for the monitoring of signal transduction dynamics and the analysis of clinical samples. Here we present a compact and highly flexible tool to read, annotate and normalize data from RPPA experiments.

Choosing appropriate algorithms to estimate and normalize the sample concentration is critical with respect to the final data quality. For this reason the RPPanalyzer package has integrated four different options to estimate the abundance of a certain target protein in all samples of interest either from replicate spots of a single concentration or from a dilution series. To calculate the concentration from dilution series three different algorithms were implemented: (1) linear approach (2) logistic approach and (3) serial dilution curve algorithm (Zhang et al., Bioinformatics 2009).

To normalize the signals according to the total protein concentration, signal intensities after staining with dyes such as Fast Green, Sypro Ruby, and colloidal gold can be used as well as signals from arrays incubated with antibodies against housekeeping proteins. Another method utilizes row normalization assuming that all measured targets are reflecting the total protein concentration of a certain sample.

Finally, data is returned as an R-object (ExpressionSet) that can easily be used for further analyses with microarray tools from the Bioconductor environment (e.g. functions of the limma package). Export of data for spreadsheet software applications (MS Excel, Openoffice spreadsheet) is also possible using the csv-file format.

A pipeline for the discovery of alternative splicing events with Affymetrix Exon Arrays

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Background. Alternative splicing is one of many processes responsible for the diversity of the proteome. This diversity is achieved by including or excluding exons during the post-transcriptional processing. This process might be impaired in cancer-cells, thus, it is of particular interest to look for cancer-specific splice variants. A microarray platform, which allows the analysis of alternative splicing on a genome-wide scale has been used. The platform has probe sets for every exon instead of having just probe sets targeting the 3' end of the gene. It contains more than 5 million probes, which allows for both, analysis of differentially expressed genes and analysis of specific splicing events between one or more biological groups, e.g. tumor vs. normal tissue.

Results. In this work the statistical challenges of identifying differential splicing patterns are addressed by using the exon profile of a gene. Low expressed genes are filtered out by using a distribution of 25.000 background probes, a linear model is fitted to each gene and as a last step an ANOVA approach is used to identify those genes with a significant alternative splicing effect.

Conclusion. The method was tested on several publicly available datasets. The first one was a spike-in dataset where it showed high specificity and sensitivity. A second dataset consisted of colon-cancer data where our algorithm found cancer-specific splicing variants that have already been validated by RT-PCR in other publications.

Fosmid Sub-genomes – HybSelect array-based targeted enrichment for Ligation-based sequencing with the SOLiD system

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Motivation:
Front-end technologies for targeted selection of genomic regions are important to efficiently utilize the high data production capabilities of next-generation sequencing platforms. We have now applied the HybSelect microarray-based technology to specifically enrich fosmid sequences in combination with SOLiD sequencing.

Resources & technologies:
As a major component of our NGFN-Plus project on 'MHC haplotype sequencing', we have established a SOLiD next generation sequencing (NGS) & data analysis pipeline. Fosmid libraries prepared from 100 human individuals from the PopGen cohort ('Haploid Reference Resource') provide the basis for the targeted selection of defined haploid fosmid sequences.

Approaches & results:
HybSelect experiments were designed to capture a defined number of fosmids from a well characterized library with 300 pooled fosmids, which had been NGS sequenced beforehand. Different experimental array designs and hybridization procedures have been tested by use of both the fosmid sequencing library and a genomic library from the same individual. The enriched libraries have been directly used for emulsion PCR and subsequent SOliD sequencing.
On average, 68.9% of reads mapped back to targeted fosmids with a ~200 fold enrichment ratio. First SNP data analyses are yet ongoing.
We demonstrate that Febit´s HybSelect array, our sub-genomic fosmid libraries and our SOLiD sequencing pipeline can be well combined to a smooth workflow to successfully enrich haploid fosmids of 40 kb size.

Statistical analysis of MeDIP-Seq data

Lukas Chavez, Jörn Dietrich, Christina Grimm, Michal Schweiger, Hans Lehrach, Bernhard Herrmann, Ralf Herwig

Max Planck Institute for Molecular Genetics

Genome-wide analysis of DNA methylation, in particular Methyl-DNA immunoprecipitation (MeDIP) followed by next generation sequencing (MeDIP-Seq), has become an important screening method for identifying potential markers for human diseases. However, it is still an issue how these data can be processed, managed and analysed in a proper way. Here, we describe the requirements and potentials of such experiments from the bioinformatics perspective. We provide an overview of different software components that are used for analysing MeDIP-Seq data, in particular with respect to read mapping, normalisation, peak finding and follow-up statistical analysis. We performed a detailed simulation of coverage and reproducibility of such experiments in order to gain the necessary depth of sequencing. We furthermore constructed a benchmark data set consisting of MeDIP and bisulfite sequencing data in order to test performances of normalisation and pre-processing procedures. The different modules of MeDIP-Seq data analysis have been implemented as a computational pipeline and are used within the IG “Modifiers of Intestinal Tumor Formation and Progression”.

High-throughput RNAi knock-down image screening of neuroblastoma cells

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To monitor chromosome segregation during cell division, chromatin condensation and fragmentation during apoptosis with time lapse fluorescence imaging, we have established several subclones of neuroblastoma cell lines stably expressing the core histone 2B tagged with GFP. We have performed a pilot RNAi screen to investigate the ability of neuroblastoma cells to uptake siRNA from solid phase, and the development of the phenotype when transfected with siRNAs. Time lapse runs are performed spanning of 48, 72 and 120 hours, respectively, with imaging every 30 minutes using GFP filter sets and a fully automated Olympus SCAN-R screening station. Furthermore, we have developed an image analysis workflow for automatic classification of cell nuclei. Our workflow comprises of segmentation, feature extraction, and classification into the classes interphase, mitosis, apoptosis, and clustered nuclei. We needed to speed up the feature extraction for which we implemented an algorithm for Haralick texture features on graphical processing units (GPUs). We are now establishing a machine learning algorithm that uses these features to distinguish phenotypic patterns of negative and positive controls.

Ultra-deep Sequencing of the small RNA Transcriptome reveals differential expression of microRNAs in high-risk versus low risk neuroblastoma

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Background: Neuroblastoma, the most common extracranial tumor of childhood, is characterized by remarkable heterogeneity in patients' clinical courses. Small non-coding RNAs, namely microRNAs, regulate gene expression and can act as oncogenes or tumor suppressor genes. Differential miRNA expression has been reported to be of functional relevance for neuroblastoma tumor biology. With the availability of next generation sequencing, the small RNA transcriptome is now accessible to unbiased absolute quantification.
Results: We analysed the small RNA transcriptome of 5 favourable and 5 unfavourable neuroblastomas using ABI SOLiD next generation sequencing. Favourable and unfavourable neuroblastomas could be separated using cluster analysis. Oncogenic miRNAs of the miR17-92 cluster and the miR-181 family were overexpressed in unfavourable neuroblastoma, although these miRNAs were also highly abundant in favourable neuroblastomas. In contast, tumor suppressive miRNAs miR-542-5p and miR-628 were moderately abundant in favourable neuroblastomas and virtually absent in unfavourable neuroblastomas. High expression of certain miR* was detected. Correlation of miRNAs with their respective miRNA* was variable. Sequence analysis revealed extensive post-transcriptional miRNA editing. Putative novel miRNAs were identified using miRdeep. Expression profiles generated by deep sequencing correlated well with real time PCR data.
Conclusions: Next generation sequencing is a valid tool for comprehensive analysis of the neuroblastoma small RNA transcriptome. Fundamental differences between the microRNA transcriptome of favourable and unfavourable neuroblastoma exists, and differentially expressed miRNAs have the potential to act as oncogenes and tumor suppressor genes. Expression of miR* and isomiRs increases the complexity of the small RNA transcriptome and awaits further functional analysis.

A long-range PCR-based targeted enrichment workflow for Next Generation Sequencing

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One of the key applications of Next Generation Sequencing is to study the genetic variation between healthy individuals and patients using whole – genome or targeted resequencing. The perhaps best-established approach for high throughput population-based sequencing of targeted intervals in the human genome is to amplify the regions of interest using long-range PCR followed by Next Generation sequencing.
In our current project we aim to resequence 10 validated disease susceptibility loci in 56 individuals (30 cases, 20 controls and 6 HapMap control samples). In order to enrich the samples for genetic diversity at the locus of interest, haplotype analyses were carried out using dense genotyping data from available genome-wide association scans. Cases and healthy controls carrying the associated risk haplotypes were preferentially selected and individuals with the remaining haplotypes were used for filling the pools. This means that patient sets that are resequenced vary between targets. Full sequence variation – i.e. in coding and non-coding genomic sequence - and individual haplotypes for these 10 disease-associated genes will be obtained. For these ten loci, more than 5500 long-range PCR amplicons have been successfully generated. This large number requires a high degree of automation and logistics. On the conference, we will show first proof-of-principle data for NOD2 and we will give a demonstration of the employed workflow.

RNA sequencing reveals unprecedented complexity of the murine intestinal transcriptome

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The transcriptome is the complete set of transcript isoforms in a cell, a tissue or a whole organism at a given point in time; observing the transcriptome is a key point in the decipherment of molecular constituents and may lead to a better insight into cellular dynamics, for example during development or disease. While to date microarray-based approaches are the method of choice to investigate the transcriptome on a genome-wide level, next generation sequencing offers a powerful alternative overcoming several limitations of hybridization-based methods. Here we show an approach to investigate the transcriptome of the murine intestine in hitherto unknown depth. Based on enriched mRNA we constructed cDNA libraries from murine small intestine and colon tissue. These libraries were sequenced on the SOLiD system leading to tens of millions uniquely mapping 35bp reads. For data analysis we established a two step matching algorithm: first we matched against an artificial sequence consisting of exons and putative splice sites; remaining reads were matched against the murine genome to identify so far not RefSeq-annotated transcripts. Thus, we found more than 18,000 covered RefSeq transcripts in the intestine, about 80,000 covered putative splice sites and several thousands of not-annotated but transcriptionally active genomic regions. These findings emphasise the usefulness of next generation sequencing technology in transcriptomics, in particular RNA sequencing indicates a far greater complexity of transcriptomes which justifies further analytic approaches in the future.

The German participation in the 1000 Genomes Project

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The international Human Genome Project has, with participation of German groups, established the reference sequence of the human genome. With the advent of next generation sequencing technologies, it has now become feasible to extend this analysis to a detailed characterisation of the genomes of individual humans, an essential basis for the discovery and understanding of the genetic variants that influence human disease. The analysis of individual genomes will provide the missing link to translate the wealth of recent association findings into an individual understanding of how the phenotypes are generated. In response to this, the 1000 Genomes Project (www.1000genomes.org) has been launched in January 2008 by a number of international centres. In August 2008 the MPIMG joint this initiative. The project is divided in a pilot and a full-project phase. Within the first period three pilot projects were designed. Under the first pilot project, researchers were sequencing 60 HapMap samples from three different populations at low coverage. The second pilot involved sequencing two trios – parents and child – of European and African descent at high coverage. The third pilot project aims to sequence 1,000 genes in 1,000 individuals at high coverage. The MPIMG participated in the first two pilot projects producing over 100 Gigabases of high quality mappable reads. Since the beginning of 2009 the socalled production phase of the project started with the aim to sequence the DNA of 1200 individuals with a coverage of 4x. This project will be key for a further understanding of genetic variation and hence have a significant importance for commercial exploitation.

nanoPCR platform: a miniaturized high-throughput PCR technology on a nanoliter scale for multiple applications in human disease research and environmental monitoring

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In life sciences there is a steadily increasing demand for miniaturized high-throughput technologies in the area of human disease research and diagnostics as well as for environmental monitoring in regard to food quality and safety. This demand stimulated efforts to develop the nanoPCR platform which is utilizable for multiple applications e.g. epigenetic methylation analysis, SNP genotyping and Salmonella serotyping.

The nanoPCR platform is based on the fast and robust PCR technique with all its inherent analytical benefits facilitating a high-throughput methodology by decreasing the assay volume to the nanoliter level. The nanoPCR platform is based on the small self-developed high-density nanoPCR chip which is 4*4 cm in size and comprising 1024 wells. Reactions are preformed in 50 to 200 nanoliters which results in increased work throughput by diminished reagent expenses as well as an essentially reduced demand of sample material. The workflow complexity is the same as for conventional PCR except that loading of the nanoPCR chip is done by non-contact nanoliter dispensing robotics. Furthermore analysis of the PCR products is accomplished by fluorescent measurements omitting standard gel electrophoresis. By means of this methodology in one nanoPCR run more than thousand simultaneous PCR reactions can be performed and analysed. The nanoPCR is based on TaqMan assays either as real-time PCR or as an end-point measurement.

In different applications ranging from gene expression studies, SNP genotyping, epigenetic methylation analysis and Salmonella serotyping the nanoPCR was applied successfully. SNP genotyping was done for SNPs associated with cervical cancer, epigenetic methylation changes were analysed in colorectal cancer samples and serotyping of Salmonella spp. was performed on food samples.

Solution-Based Enrichment of Genomic Loci for Massively Parallel Sequencing on the SOLiD™ System.

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The identification of genetic variants and mutations associated with human disease requires the development of a robust and cost-effective approach for systematic resequencing of candidate regions in the human genome. The SOLiD™ System acquires tens of gigabases of mappable sequence within a single run, allowing for accurate resequencing of large genomes. When combined with the Agilent SureSelect™ Target Enrichment System, the ultra-high throughput of the SOLiD™ System facilitates deep sequencing of target genomic regions of interest. The SureSelect™ method extracts target regions from genomic DNA by hybridization to in-solution biotinylated cRNA probes, or “baits. ” The target enrichment workflow is streamlined to incorporate this enrichment step after libraries are constructed. Post-enrichment material is amplified and used directly for downstream steps including emulsion PCR and sequencing on the SOLiD™ System. Sequence capture of a 2.7 Mb region from Yoruba DNA resulted in 700-fold enrichment with 65% of reads mapping uniquely to the target region, as compared to 0.1% for an un-enriched control. Due to the inherent scalability and potential for automation of SureSelect™ in-solution enrichment, the coupling of this method with the SOLiD™ System platform provides a useful solution for targeted resequencing applications.

Single-step procedure for the isolation of proteins at near-native conditions for proteomic analysis with antibody microarrays

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The process of extracting comprehensive proteome representations is a crucial step for many proteomic studies. While antibody microarrays are an evolving and promising methodology in proteomics, the issue of protein extraction from tissues for this kind of analysis has never been addressed. Here, we describe a single-step extraction buffer for the isolation of proteins from mammalian tissues under native conditions in an effective and reproducible manner. Protein was extracted from cell lines BxPC-3 and SU.86.86, rat organs (pancreas, liver, heart and lung) and human pancreatic cancer tissues using several buffer systems that contained individual non-ionic or zwitterionic detergents in comparison to commercial extraction buffers. Also, detergent combinations were used that included at least one polymeric phenylethylene glycol, a long-chain amidosulfobetaine, cholate and a zwitterionic detergent. Extracts were analysed for protein quantity and quality. The detergent cocktails exhibited superior extraction capacity. Additionally, they demonstrated a substantially higher recovery of membrane and compartmental proteins as well as much better preservation of protein functionality. Also, they did not interfere with subsequent analysis steps such as labelling. In Western blot and antibody microarray assays, they out-performed the other buffer systems, indicating that they should also be useful for other types of proteomic studies.

Establishment of a whole transcriptome data analysis workflow and cognate modules for quality control and quantitation using the SOLiD ™ 3 system.

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Ultra high-throughput sequencing of RNA (RNA-Seq) has shown great promise for the measurement of transcriptional activity at a single nucleotide resolution. The tradeoff for such a powerful technology is management of the voluminous resultant data and what analysis workflows are appropriate for answering specific biological questions. We will present an analysis workflow for assessing quantitation and characterization of millions of RNA sequence reads generated by the SOLiD ™ 3 system. We have established logical bioinformatics workflow consisting of a combination of read mapping to a reference genome after specific filtering of overrepresented RNA species combined with several summarization modules. Included are modules that effectively report QC information in the form of reproducibility measures, filter mapping statistics as well as quantitation and per base coverage of well known spike in controls and housekeeping genes. Downstream modules report a per nucleotide transcriptional index across each chromosome which may be used for comparing run to run reproducibility and serve as an estimator of differential expression for annotated segments of the genome. In addition, saturation reports describe the fraction of annotated RNAs that are detected per sample per million reads in common reference RNA sequence databases including RefSeq and the more comprehensive AceView. This workflow has allowed for facile interpretation and faster turnaround when evaluating transcriptional activity and QC information from a given sample RNA.

Next Generation Sequencing (NGS) platforms at the Cologne Center for Genomics

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NGS offers opportunities promoting projects inaccessible before in medical genetics, e.g. the sequencing of tumor transcriptomes, the fast and cost-effective analysis of hundreds of candidate disease genes simultaneously or the search for rare disease causing variants on a genome wide scale.
Since NGS needs experts both at the bench and on the bioinformatics side we started to build up two different platforms at the Cologne Center for Genomics offering expert advice, operating service and advanced bioinformatics both for Illumina's GAII and Roche's 454 sequencing machines. We already conducted successfully de novo sequencing and DNA re-sequencing, and transcriptome, ChiP, micro- and t-RNA sequencing on a GAII Illumina instrument. On the 454 sequencer we performed amplicon sequencing and de novo transcriptome and DNA sequencing for projects requiring longer read lengths then obtainable via the Illumina instrument. Currently we are testing different enrichment techniques (NimbleGen, Febit, RainDance, and Agilent) on both platforms for the targeted analysis of candidate genes and whole-exome enrichment to compare performances and optimal fields of application. Our bioinformatics experts are setting up analysis pipelines for the different data sets tuned for the specific questions to be answered in close cooperation with our clinical partners.
We feel confident to solve all the upcoming questions concerning these new and highly sophisticated NGS technologies and to offer interesting and successful partnerships in scientific cooperations.

Analysis of protein-protein interactions by the use of interfering intrabodies

Tonio Schütze, Susanne Weber, Linda Hallen, Theam Soon Lim, Josmar Langner, Silke Wehrmeyer, Ute Nonhoff, Markus Ralser, Hans Lehrach, Sylvia Krobitsch and Zoltán Konthur

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Over the last few years, worldwide research has clearly demonstrated that abnormal protein-protein interactions contribute to the pathogenesis of numerous human disorders such as cancer or neurodegenerative disorders. To determine whether a protein-protein interaction is implicated in pathogenesis, a novel approach for obtaining intrabodies, which specifically interfere with protein-protein interactions, has been developed. This technology is a valuable and powerful tool for our studies, because it allows interference of a particular protein function/protein interaction. Our work is based on a combination of in vitro / in vivo ligand screening methods using phage display and the yeast-2-hybrid system. The great benefit of this technique is the systematic weed out of non-inhibitory binder molecules at an early stage resulting in a manageable number of highly specific binder molecules with functional activity. This may allow the validation of single biological processes without affecting any additional function of the gene product.
Thus, this technology will be a valuable and powerful tool for gaining insights into pathways underlying human disorders as well as the molecular function of individual proteins.

Analysis of Alternative Splicing by next generation sequencing

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***These authors contributed equally to this work**

Alternative splicing of pre-messenger RNA molecules of multi-exon genes can produce a variety of transcript isoforms playing specific biological roles and whose respective expression levels are regulated in time and space. However, the repertoire of mammalian alternatively spliced transcripts and their regulation are still poorly known. Second generation sequencing is now opening unprecedented new routes to address the analysis of entire transcriptomes. Here, we develop methods allowing the prediction and quantification of alternative splicing events from RNA-Seq data. Analysing two human cell lines, human HEK293T and Ramos B cells, we could detect alternative splicing occurring within a cell type and between cell types. We predicted 2,499 alternative splicing events in 2,070 genes for HEK and 1,775 events in 1,532 genes for B cells, respectively. Further, we estimated the proportions of transcript isoforms, for 830 genes in HEK and 640 genes in B cells, solely based on exon expression levels and data showed that these predictions correlated well with information originating from junction reads.. A direct comparison with exon arrays indicated improved performances of RNA-Seq over microarrays in the prediction of skipped exons. Altogether, the methods described here adress different aspects of alternative splicing using the density of reads in exons. Importantly, we showed that combining the use of these methods complements the information given by junction reads offers an increased analysis power, in particular for typical datasets limited to a few sequencing lanes.

Structural variations analysis with SOLiD next generation sequencing technology: Coverage requirements, resolution and biological interpretation.

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Max Planck Institute for Molecular Genetics

Currently available next generation sequencing platforms have an enormous potential to bring changes in genetic and biological research and to enhance our understanding of complex genomic diseases such as cancer. We use the SOLiD platform for whole genome re-sequencing since – based on synthesis-by-ligation sequencing and its di-base encoding – the platform enables an enormous parallel throughput with high data quality. Despite a relatively short read length of 50bp the output of uniquely mappable data doubled within the last months to 20-24 Gb per run.
Copy number variation (CNV) analyses and mutational analyses are shown for two colorectal cancer cell lines, SW480 and SW620. In particular, we present a comparison between the recently released SOLiD CNV tool 1.0 and the public available CNV-seq method (Xie et al. 2009) and describe resolution, coverage requirements and influence on biological findings.

Comparison of three high-resolution oligonucleotide arrays for the detection of copy-number aberrations

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Local deviations from the diploid copy number status occur frequently, both in healthy individuals and people suffering from Medelian or complex genetic diseases like autism, epilepsy or schizophrenia. There is growing evidence that such copy-number aberrations (CNAs) are involved in the etiology of many forms of mental retardation, and they also play an important role in tumorigenesis. Classical cytogenetic techniques like G-banded chromosome analyses are limited with respect to the resolution power. In recent years they have been increasingly complemented and replaced by array-based technologies, namely BAC arrays and oligonucleotide arrays.
We have compared the power of three high-resolution oligonucleotide arrays to detect CNAs in eight challenging human patient samples. The 3 array types differ in the length and number of the oligonucleotides used, as well as in the amount, distribution, and type of DNA variation to be analyzed (SNP and/or CNA). Intriguingly, the Affymetrix SNP 6.0 array, which represents probes for the interrogation of 906,600 SNP markers and additional 946,000 copy number probes, revealed a homozygous (and heterozygous) deletion as small as 11 kb affecting only three exons of two adjacent genes, thereby directly pointing to the gene LRRC50 mutated in patients with a novel variant of primary ciliary dyskinesia. We will present these and additional data generated by the Affymetrix SNP 6.0 array, the Affymetrix Cytogenetics Array, and the Agilent 1 M CGH Array using the same set of samples for direct comparison of performance and discuss the strengths and limitations of each platform.

Charting Gene Regulation Networks of Chromosome 21 Transcription Factors using RNAi and ChIP-seq

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The regulation of gene expression plays a major role in the orchestration of complex mechanisms underlying mammalian development, differentiation, and homeostasis. Studies in model organisms showed that gene dosage effects involving transcription factors (TFs) often have deleterious effects. In trisomy 21, resulting in Down syndrome, over-expression of the TFs encoded by human chromosome 21 (HSA21) is likely to significantly alter the mRNA levels of a number of target genes, some of which can have a contribution to the phenotype. Understanding these transcriptional regulatory networks requires a global identification of the TF binding sites in the genome.

Our strategy to identify TF target regions genome-wide, in the context of the natural nuclear environment, is to perform chromatin immunoprecipitation coupled with second-generation DNA sequencing (ChIP-seq). We have developed a highly specific ChIP-seq protocol and applied it to four HSA21 TFs, namely BACH1, GABPA, PKNOX1 and ERG, which are endogenously expressed in HEK293 or CCRF-CEM cells. Sequencing of the ChIP DNAs delivered >10 million reads for each TF. Computational analysis identified regions enriched in reads that served as basis for annotation of all potential TF target genes.

We present an overview of the workflow and discuss the genome-wide landscapes of the analyzed TF binding sites as well as the biological functions of their multiple target genes. Data is being integrated with data from corresponding RNAi experiments coupled to expression profiling, giving an impression of the consequences on target gene expression resulting from knock-down of the HSA21 TFs. Taking as example the redox-regulatory factor BACH1, we show that the combination of ChIP-seq and RNAi data can be used to gain detailed insights into the orchestrated course of expression changes of BACH1 target genes, resulting in metabolic adaptations important for maintainance of cellular homeostasis.

Finding the needle in the haystack: towards building a bioinformatic pipeline for the systematic analysis of whole exome sequencing data

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Recent advances in high-throughput sequence capture and massively parallel sequencing technologies now enable researchers to quickly generate large amounts of sequence data in the search for disease modifying genetic variants. While the generation of these data is relatively straightforward on the commercially available next-generation sequencing instruments, there currently exists no integrated bioinformatic approach for the detailed analysis of the resulting data. We have recently begun "whole exome" sequencing in probands of 10 early-onset Alzheimer's disease (AD) families with the aim of identifying novel disease-causing mutations. Sequence data was generated using microarray-based sequence capture of ~180,000 coding regions followed by sequencing on a Roche/454 GS FLX system. After initial data analysis, five patient-control pairs were retained for in-depth sequencing. To date, the project has resulted in more than 15 Gb of raw sequence, 95% of which could be uniquely mapped to the genome, yielding over 150,000 non-redundant sequence variants across the five index patients. We then systematically analyzed these data by integrating over a dozen different software tools (self-written and publicly available) to evaluate and compare our sequences to data generated both in our group (500K Affymetrix SNP-Chip genotypes), and available in the public domain (e.g. NCBI's dbSNP, Ensembl, or the "1000 Genomes Project"). To this end, we particularly focused on variant frequency, levels of brain expression, evolutionary conservation, potential protein-interactions, and in silico phenotype predictions of non-synonymous variants. This multi-pronged analysis process resulted in less than 250 rare sequence variants, which are currently tested for disease segregation within families using conventional Sanger sequencing. At the meeting, we will present details of the bioinformatic stategies and results of our project, and discuss some of the most prominent pitfalls along the way.

National Genome Research Network

Poster presentation abstracts

Symposium VI

Transfer from Genomics to Application

Development and validation of new diagnostic, preventive and therapeutic tools for the prevention of cardiovascular diseases and disorders (CVD) in chronic kidney disease (CKD)

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The consortium applies the novel tools “proteomics, peptidomics and metabonomics, and genotyping”, which allow assessing the complete transcription and translation of the genomic capital to elucidate the genetic and physiological background of CVD in CKD patients. This approach is focused on human samples i.e. tissues, cells and body fluids as humoral targets are altered in CVD of CKD patients. NTCVD applies "forward genetics" from phenotype to gene to remedy the causes of the enormously accelerated cardiovascular morbidity and death in CKD (stage 3-5) and to develop novel diagnostics and therapeu-tics, based on molecular genotyping and phenotyping. This is done (A) by elucidating the role of recently identified mediators relating to CVD in CKD by using bioassay approaches and pattern analysis of CKD patient samples, and (B) by the identification of yet unknown mediators. Findings and results will be translated directly into new diagnostic and therapeutic devices for the prevention and treatment of CVD in CKD by collaborative efforts of the industry partners (one SME, one industrial company) within NTCVD.

Identification of a new angiogenic factor

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The secretion of angiogenic factors by vascular endothelial cells is one of the key mechanisms of angiogenesis. Here we report the isolation of a new potent angiogenic factor from the secretome of human endothelial cells. Beside a strong angiogenic effect on the yolk sac membrane and the developing rat embryo itself, the factor increased the prolif-eration rate of endothelial cells and, in the presence of PDGF, of vascular smooth muscle cells. The factor stimulated the migration rate of endothelial cells via P2Y2-receptors and increased the ability of endothelial cells to form capillary-like tubes. This effect was additive to that of PDGF. Endothelial cells released Up4U after stimulation with shear stress. Mean total plasma of this factor concentrations of healthy subjects (N=6) were sufficient to induce angiogenic and proliferative effects. In conclusion, the new factor is a novel strong human endothelium-derived angiogenic factor.

NT-CVD-Register: a prospective, longitudinal approach identifying and correlating different biomarkers with clinical cardiovascular risk assessment and outcomes in patients with chronic kidney disease

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Background: Cardiovascular diseases (CVD) are the leading cause of death in western countries. In patients with chronic kidney disease (CKD) cardiovascular morbidity and mortality is even higher than in the general population. The mechanisms why CKD-patients develop CVD much earlier and at much higher frequency are not clearly understood, but accelerated atherosclerosis and inflammation-related vascular damage seem to play a critical role.

Aim of the study: To identify known and unknown profibrotic and proinflammatory biomarkers which are correlated with and potentially mediate CVD in patients with CKD.

Methods:

A total of 300 subjects with moderate to severe CKD including subjects on hemodialysis and a control group of subjects with normal renal function will be included in the study. At baseline (BL), 12 and 24 month after BL cardiovascular and renal function will be characterized clinically and non-invasively including pulse wave analysis, ankle-brachial-index, electro- and echocardiography, exercise-testing with determination of cardiac index and oxygen uptake by inert-gas-rebreathing (Innocor®). Classical cardiovascular risk factors, cardiovascular history and events will be documented. Urine and blood samples will be collected to determine known proinflammatory and profibrotic biomarkers like NGAL/lipocalin-2, resistin, MCP-1, Osteopontin, PAI-1, KIM-1, TIMP-1 and others as well as to identify yet unknown biomarker by mass spectrometry, other bioassays and pattern analysis will be performed.

Current Status: 130 subjects (66 with CKD and 64 with normal renal function) with and without history of CVD have yet been included in the study and have finished the baseline visit. As soon as the first subjects have passed the 12 month visit analysis of biomarker and analysis of their association with CVD will be performed.

This study is conducted in the context of the NGFN-Transfer project “NT-CVD - New Tools for the Prevention of CVD in CKD”.

Cognition in chronic kidney disease - results from NT-CVD-Register

Kognition bei chronischer Niereninsuffzienz - Ergebnisse des NT-CVD-Registers

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Mit dem Altern der Gesellschaft, der steigenden Prävalenz an Diabetes, Hypertonie und anderen kardiovaskulären Erkrankungen ist Niereninsuffizienz in den letzten Jahren ein größeres Gesundheitsproblem geworden, und die Prävalenz von Niereninsuffizienz wird voraussichtlich weiter ansteigen. Frühere Studien zeigten kognitive Defizite bei niereninsuffizienten Patienten. Aufgrund der hohen Komorbidität von Niereninsuffizienz mit kardiovaskulären Erkrankungen konnte bis jetzt noch nicht untersucht werden, inwiefern Niereninsuffizienz per se zu kognitiven Defiziten beiträgt. Dies wurde in der hier vorgestellten Studie untersucht. Patienten mit chronischer Niereninsuffizienz Stadium 3-5 (nach KDOQI) und nierengesunde Kontrollen jeweils mit kardiovaskulären Risikofaktoren bzw. manifesten kardiovaskulären Erkrankungen nahmen an der Studie teil. Dies ermöglichte die Kontrolle des Einflusses von kardiovaskulären Komorbiditäten auf kognitives Vermögen. Neuropsychologische Untersuchung erfasste folgende Bereiche: psychomotorische Geschwindigkeit, kognitive Flexibilität, verbale Flüssigkeit, Gedächtnis sowie visuokontruktives Vermögen. Angst- und Depressionssymptomatik wurde mit einem kurzen Fragebogen erfasst. Die Störungsfaktoren wie Tremor und beeinträchtigtes Sehvermögen wurden ebenfalls kontrolliert. Das Stadium der Niereninsuffizienz wurde nach KDOQI (siehe unten- falls nähere Erläuterung angegeben werden soll) mit Hilfe der geschätzten glomerulären Filtrationsrate (eGFR) bestimmt, die mit der Modification of Diet in Renal Disease Formel berechnet wurde. Die vorläufigen Ergebnisse werden dargestellt.
Die Studie wird im Rahmen des NGNF-Transfer Projekts “NT-CVD - New Tools for the Prevention of CVD in CKD” durchgeführt.

Subgenome fractionation of up to 12 samples using only one microarray for downstream HT-Sequencing

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HT-Sequencing is a powerful tool for the identification of genomic variations, which play a significant role in the development of all kind of disease. However, sequencing the whole genome is still far too cost intensive. High depth of coverage is necessary in order to detect sequence variations. Therefore subgenome fractionation is an important tool to enrich for regions of interest.
Here, we report the microarray-based genomic selection (MGS) for enriching genes involved in cardiomyopathies and arrhythmias. We selected the exons and splicing signals of 2000 genes (3.1 Mb) and used Nimblegen's microarrays (385k) to enrich the DNA of patients with cardiomyopathies and arrhythmias and healthy people. 454-sequencing results showed that 75% of the mapped reads are within the target region. The enrichment (coverage of the target regions versus the coverage of the non-target regions) is 1345. The average depth of coverage shows a relatively homogenous distribution.
Additionally, we are able to use multiplex identifier (MID) in order to enrich the DNA of up to 12 patients on the same microarray in parallel, enabling us to predict deletions or insertions in individual patients. We have developed a protocol to use the identifiers in combination with 454 sequencing and Illumina sequencing platform.
Currently we are establishing procedures, combining target enrichment of promoters and nanobisufite treatment in combination with next-generation sequencing. This process will allow for detailed methylation analysis of defined regions within the genome. It can be used in place of MeDIP chip analysis, which often is hampered by low signal to noise ratios, or complexity reduction set-ups (Meissner et al., Nature 2008). One of our first projects will address promoter analysis of genes involved in glioblastoma.

Whole genome and transcriptome amplification in large biobanks

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Biobanks are a key resource in unravelling the molecular basis of diseases, identification of new targets for therapy and improvement of attribution in drug discovery and development. The scientific trend in biobanking shows the need for stable techniques for amplification of biomaterials, which can be used for samples stored under very different conditions. The focus of the project is the standardisation and validation of the innovative techniques of whole genome amplification (WGA) and whole transcriptome amplification (WTA) in the context of biobanks. A general standardized protocol for WGA and WTA procedures that use Phi29-DNA-polymerase in biobanking will be developed.The major aims of our project are:
1. To establish standardized WGA protocols for large biobanks
2. To develop standardized WGA tools to recover genomic DNA, which is in plasma or serum samples and from FFPE- tissue or blood spots
3. To optimize the WGA procedure by extensive quality control measures of WGA products
4. To develop and establish WTA of large biobank samples
5. To optimize WTA procedures by extensive quality control of WTA products
Furthermore, the concept of the project is to transfer the results of WGA and WTA solution to national and international organisations in the field of biobanking. The development of the proposed, innovative and specialized tools and customized solutions will help to expand and secure biobanks.

Microspot immunoassays for biomarker discovery in plasma of breast cancer patients

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Over the last years different protein microarray platforms emerged as tools for biomarker discovery. They allow the simultaneous determination of various parameters from a minute amount of sample within a single experiment. The potential use of biomarkers in breast cancer includes aiding early diagnosis, determining prognosis and predicting response or resistance to specific therapies. The ease with which blood can be sampled from patients makes it a logical choice for biomarker applications.
Two microspot immunoassays were developed and validated for the determination of biomarker signatures in the plasma of breast cancer patients. An 8-plex microspot immunoassay for the quantification of ERBB receptor family ligands (amphiregulin, betacellulin, epidermal growth factor, heparin-binding EGF-like growth factor, neuregulin 1, and transforming growth factor alpha) as well as the ligand of the MET receptor (hepatocyte growth factor) and the vascular endothelial growth factor. In addition, a 4-plex microspot immunoassay quantifies the extracellular shedding products of ERBB1, ERBB2, ERBB3, and MET receptor. Both, 8-plex and 4-plex microspot immunoassay, will be used for the determination of target protein concentrations in more than 100 plasma samples from breast cancer patients collected at the time point of biopsy. Furthermore, the experimental data will be compared with clinical data.

Quality management and standardization within the Program of Medical Genome Research – NGFN-Plus and NGFN-Transfer

Stefan Wiemann, Working group Quality Management and Standardization in the NGFN

Researchers representing the different clinical and experimental research topics of the NGFN and who are driving forces of quality management (QM) in the respective projects have formed the NGFN workgroup on QM & Standards (AG-QM). This group works out strategies to optimize the quality and reproducibility of data management and of research that is performed within NGFN, and to develop common standards for efficient data exchange. The consensus is then transferred into the individual NGFN projects and is implemented there. Standardized protocols for genomics experiments and other relevant processes in the NGFN have, in part, been established and are reviewed for dissemination also to the public through the web pages of the quality management platform of the NGFN.

After having completed a survey on the most urgent needs for progress in QM within the NGFN, future progress will be mostly made in dedicated working groups and workshops focusing on individual and specific aspects of relevance to the community. Such workshops have been organized in the past, for example, on the management and use of clinical data, and on next generation sequencing. Additional topics will be implemented to further stimulate and drive harmonization of data annotation to permit data exchange and integration. The respective quality standards mostly follow international guidelines, if they exist. The AG-QM and the relevant structures within the individual projects are tightly connected to activities outside the NGFN, to enhance the impact of this group and of the NGFN having at the national and international levels.

Targeting Hyaluranidase2 to disrupt the HA/CD44/Met complex in breast cancer therapy

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Failure of cancer therapeutic drugs is mainly associated with the bypass of targeted oncogenic signalling pathways. A number of studies aim at targeting such bypass processes specific to cancer cells. Among candidates which could mediate such bypass processes is Hyaluronan (HA), a major glycosaminoglycan in the extracellular matrix, whose expression is tightly linked to tumour progression. HA and its specific receptor CD44 modulate the interactions between growth factors and their receptors, leading to tumour progression and metastasis.

In this study, we examine the effect of Hyaluronidase2, a HA degrading enzyme, on breast cancer cell progression and growth factor signalling. We show that Hyal2 siRNA could abrogate not only HGF induced Met activation, but also its downstream effects, e.g. Erk and Akt phosphorylation, which mediate cell growth and survival. In addition, in non-tumorigenic breast epithelial cells, as well as in invasive breast cancer cell lines, level of Hyal2 knockdown clearly correlates with increase in CD44 transcript levels.

Taken together, our results suggest that in future Hyal2 inhibitors might be interesting potential drugs for cancer treatment. Using a 3D cell culture system which closely mimics the breast tumour tissues, we will evaluate the consequence of CD44 upregulation in Hyal2 knockdown cells using a specific CD44 therapeutic antibody. Measurements will be performed in non-tumorigenic breast cells, invasive breast cancer cell lines and in cancer stem cell-like enriched cell populations. To validate Hyal2 as a potential target for breast cancer therapy, we will correlate its expression in tumour samples with the expression and activation of signalling proteins, clinical relevant histological findings, as well as with the appearance of circulating tumour cells.

Keywords: Breast cancer, HA/CD44; HGF/Met; Hyal2

A new diagnostic approach to identify variable somatic tumor mutations

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A variety of mutations have been described for NSCLC tumors and the occurrence of some of them already impact therapy. The diagnosis of these variants is not trivial, since many of them are not simple SNPs at defined positions; rather a multitude of possible variations including deletions within mutation hot-spot regions is observed. Additionally, genotyping approaches are often hampered because tissue obtained by biopsy provides only a small amount of raw material, which contains sometimes only a few tumor cells accompanied by predominantly healthy tissue. The perfect diagnostic procedure would require a highly specific (excluding SNP-genotyping strategies), highly sensitive (excluding Sanger-sequencing) and fast and cost effective (excluding NGS) technology.
We established assays of high usability in a clinical setting using conventional Pyrosequencing®. We succeeded with assay set-ups for the 5 most common mutation "hot spots" in the EGFR exon 19-21 and the KRAS exons 2 and 3. For all five fragments linearity tests were carried out and numerous tumor cell lines and primary tumor samples were analyzed. Our assays are sophistically designed with respect to primer position and dispensing order to be highly specific for all known variants in the range known to be frequently mutated. Also some additional variants could be identified that have not yet been published before underlining the high potential of Pyrosequencing® for variant discovery in addition to genotyping. The linearity tests showed the assay to be sensitive enough to detect up to 5% of cells carrying the mutation. The diagnostic process is very cost-effective, fast, and flexible regarding the number of samples.
Due to the highly reproducible and quantifiable results, we recommend the use of this technique in diagnosis and to expand the underlying strategies also in additional applications of genotyping somatic mutations.

In situ protein tagging for proteome analysis in mouse embryonic stem cells

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Remarkable progress in human genetics has identified mutations in numerous genes that are linked with disease. However, the limitation of these discoveries is that genetics only identifies individual genes without placing them into pathways. To achieve this, it is essential to identify the physical relationships of these genes to other genes by proteomic mapping under physiological conditions. Towards this end, we have developed an in situ protein tagging strategy based on recombinase mediated cassette exchange (RMCE) in mouse embryonic stem cells (mESCs) harboring gene trap induced multipurpose alleles of individual genes. Several ESC lines with suitable gene trap insertions in disease genes were recovered from the existing gene trap resources (GGTC and EUCOMM) and subjected to RMCE using an incoming hygromycin-P2A-LAP tag cassette defined as a portable exon by upstream splice acceptor (SA) and downstream splice donor (SD) sites. Since the portable exon is flanked by heterotypic Frt/F3 recombinase target sites (RTs) that are identical to the RTs inserted into the gene trap loci, the incoming construct can effectively recombine with these loci in presence of FLPo recombinase. Accordingly, selection for exchange cassette insertions in hygromycin yielded correctly recombined ESC clones with an efficiency of seventy percent. In these clones, the portable exon was correctly spliced to the endogenous exons of the trapped gene, thereby leading to the expression of LAP tagged versions of the wild type proteins. Subcellular localization the LAP-tagged proteins closely mirrored the localization of the corresponding wild type proteins. Mass spectrometric analysis of immunoprecipitates identified several known and unknown interaction partners in each case. Overall, the experiments confirm the suitability of the in situ protein tagging methodology for high throughput proteomic mapping of proteins expressed in trapped ESC lines.

Thiopurine Methyltransferase Genetics Is Associated with Treatment Outcome and Hepatic Toxicity in Pediatric Patients with Acute Lymphoblastic Leukemia: a Report from the ALL-BFM Study Group

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Thiopurine methyltransferase (TPMT) is a key enzyme in the metabolism of thiopurines and underlies phenotypically relevant genetic variation. Heterozygotes or homozygotes for TPMT genotypes conferring lower enzyme activity demonstrate thiopurine drug metabolic patterns distinct from those of TPMT wild-type individuals. Here we report on a prospective evaluation of the role of TPMT genetics for survival and treatment-related toxicity in a cohort of 814 pediatric ALL patients. These 814 patients represent 85% of the entire patient population (n=956) enrolled in multi-center trial ALL-BFM 2000 from October 1999 to September 2002. Our analysis revealed 755 (92.8%) patients with TPMT wild-type, 55 (6.8%) with a heterozygous, and 4 (0.5%) with a homozygous variant genotype (*2/*3A, *3A/*3A [n=2], *3A/*11), respectively. Genotype frequencies were in Hardy-Weinberg equilibrium. Allele frequencies were as follows: TPMT*1 = 96.12%, TPMT*2 = 0.25%, TPMT*3A = 2.95%, TPMT*3C = 0.56%, TPMT*9 = 0.06%, and TPMT*11 = 0.06%. Patients (n=55) heterozygous for allelic variants of TPMT conferring lower enzyme activity demonstrated significantly better event-free survival (EFS) and a lower relapse rate compared to homozygous wild-type patients (n=755) (six-years pEFS; heterozygotes vs. wild-type, 95% (SE 3%) vs. 84% (SE 1%), p(log-rank) = 0.04; p(point estimate difference) = <0.001, relapse incidence at six years, 4% (SE 3%) vs. 12% (SE 3%), p = 0.07). While TPMT heterozygotes did not demonstrate statistically significant differences when their standard toxicity data were compared with wild-type patients for 6-MP-containing treatment phases, they had an increased risk of developing hepatic veno-occlusive disease associated with a two-week exposure towards 6-TG given during re-intensification. In conclusion, TPMT genotyping may contribute important information for clinical decision making in childhood ALL.

Proteome analysis of PARK6 patient fibroblasts reveals pathological Unfolded Protein Response

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Loss-of function mutations in the PARK6 and PARK2 loci encoding the PTEN induced putative kinase 1 (PINK1) and the E3 ubiquitin-ligase PARKIN cause autosomal-recessive familial variants of early-onset Parkinson's disease (PD) (Valente et al. 2004, Kitada et al. 1998). Both proteins are involved in mitochondrial dynamics (Clark et al. 2006, Park et al. 2006), autophagy (Narendra et al. 2008, Dagda et al. 2009) and proteasome impairment (Xiong et al. 2009). But the precise physiological functions of PINK1 and PARKIN are still elusive. Both proteins act cytoprotective against cellular stressors such as oxidative and ER stress, an important finding in view of the established role of environmental factors as causes of PD.
We report on changes in protein expression and pattern in human primary skin fibroblasts from 3 patients homozygous for the loss-of-function mutation G309D-PINK1 in contrast to age-matched controls by two-dimensional difference-in-gel (2D-DIGE) and western-blot (WB), with outlook to proteome analyses in compound heterozygous V56E/C212Y-PARKIN siblings.
The most interesting proteins revealed by 2D-DIGE were galectin-1 and a post-translationally modified isoform of protein disulfide isomerase A3 (PDIA3). Galectin-1 has a role in regeneration, was previously reported to be dysregulated in the substantia nigra of sporadic PD patients (Werner-CJ et al 2008) and is implicated in axonal spheroid and skin changes in motor neuron disease (Kato-T et al 2005). PDIA3 upregulation has been observed in yeast models of PD (Yeger-Lotem-E et al 2009), is part of the unfolded protein response (UPR) and is a sensitive indicator of ER stress. Basal increase of PDIA3 expression was observed in WB, but not by qPCR. The capacity to enhance PDIA3 further by tunicamycin treatment of fibroblasts was limited compared to control cells.
Ongoing research includes validation of differential proteins by quantitative MS or WB. The study will be extended on fibroblasts from 3 PARK2 cases.

Multiclass Diagnosis of Pancreatic Cancer: Accurate Distinction between Different Types of Pancreatico-Biliary Tumors using Specialized Diagnostic Arrays

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Both the timely detection as well as the accurate differential diagnosis of pancreatic cancer remain exceedingly difficult with currently available diagnostic means. We have previously designed a specialized 588 feature cDNA array for pancreatic cancer differential diagnosis. In a proof-of-principle study, we demonstrated that molecular analysis of biopsy material using this array is suitable to distinguish between PDAC and non-malignant processes of the pancreas with high diagnostic accuracy. We have now extended this work to the analysis of additional types of pancreatico-biliary tumors in order to develop a comprehensive clinical tool providing accurate differential diagnosis of the clinically most relevant tumor entities. Analysis of the total set of 156 clinical samples using linear support vector machines resulted in clear separation of the five different diagnostic entities. All individual samples were re-classified with 100 % accuracy. In order to stringently examine the general performance of the system, the data set was repeatedly randomly divided into independent training and test sets, each time training the classifier with 90 % of the data and testing on the remaining 10 % of samples, respectively (10 x 10fold cross-validation). The resulting generalized class-wise diagnostic accuracies ranged from 81 to 92 % for the different tumor entities, thus clearly outperforming conventional diagnostic procedures.
In order to further facilitate routine clinical use of this diagnostic system and to take advantage of the superior sensitivity and robustness of RealTime PCR technology, we have in close collaboration with Applied Biosystems GmbH extracted from these data a core set of 88 diagnostic genes to create a standardized TaqMan® Low Density Array. The diagnostic performance of this microfluidic card-based system is currently being evaluated within the framework of the NGFN Integrated Genome Research Network “PaCa-Net”. First results will be presented.

The Pacanet-iCHIP System - virtual biobanking in NGFN-Plus

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Biobanks are crucial for the characterization and regular collection of samples in the context of sustainable storage and valid distribution. This new virtual biobank (2009) forwards the exchange of biomaterial within Pacanet as a decisive factor.

The Pacanet-iCHIP biobank hosted at the DKFZ covers all relevant information for the facilitation of the biomaterial exploitation and the exchange between institutes. Annotation comprises e.g., histological-related information, the types of material, the corresponding immuno-histological images, diagnosis, and information about the validation and transfer process.

The biobank registration of patient materials is performed under the responsibility of the single clinics. Clinical and histological information is aligned by the European Pancreas Center in Heidelberg. All information is pseudonymized by the use of data protection instruments developed at the telematic platform for medical networks (TMF). Only samples passed the expertise advice in Kiel are approved for further applications.

The establishment of this virtual biobank system succeeded easily because of already available methods and the highly flexible database structure of iCHIP, the well-known integrative platform for the various kinds of experiments in the fields of molecular biology, cell biology and clinic.

This preliminary work leads to a positive phenomenon for our actual and future partners. Enhancements and specifications in biobanking can be realized within short time frames and without extended development costs.

Validation of automated RNA extraction for gene expression analysis in formalin fixed paraffin-embedded needle-core biopsies of prostate tissue

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Multiple molecular events characterize prostate cancer development and progression. Gene expression analysis offers a significant potential as a diagnostic, prognostic, and predictive tool, but requires high quality RNA. Formalin-fixed, paraffin-embedded (FFPE) prostate needle-core biopsies are the most common specimen available for molecular analysis on prostate tissue after diagnostic histopathological examination. Unfortunately, RNA extracted from FFPE tissue suffers from strand breakage and cross-linking and results in degraded nucleic acids of poor amplifiability. Additionally the small quantity of tissue and the large quantity of paraffin from the needle-core biopsies makes RNA extraction more difficult. Therefore we optimized and adapted the RNA extraction protocol using the RNeasy FFPE Kit to get high reproducible RNA yields of FFPE needle-core prostate biopsies.

We determined the performance analyzing total and amplifiable RNA yields as well as inhibition of real-time polymerase chain reaction. Further we implemented a deparaffinization at high temperature and optimized the protocol concerning lysis time and nucleic acid binding to increase RNA yield. To minimize user interference the manual protocol was transferred to the QIAcube, a platform that allows spin column based RNA extraction without change of purification chemistry. The automated process was analysed for intra- and inter-run variability and was validated in a set of 48 clinical samples.

The automated protocol generates an average total RNA yield of 450 to 650 ng per FFPE prostate biopsy section. The average amplifiable RNA (90bp fragment) yield is 30 to 65 ng/section. The automated RNA-purification method shows high reproducibility and is well suited for RNA analyses with archived FFPE material, and improves the utility of FFPE prostate needle-core biopsies for analysis of RNA-based biomarkers.

Comparison of one-color and two-color microarray data for classification of clinical endpoints

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Microarray-based prediction of clinical endpoints may be performed using either a one-color approach reflecting mRNA abundance in absolute intensity values or a two-color approach yielding ratios of fluorescent intensities. In this study, as part of the Microarray Quality Control (MAQC-II) project, we systematically compared the classification performance resulting from one- and two-color gene-expression profiles of 478 neuroblastoma samples. In total, 196 classification models were applied to these measurements to predict four clinical endpoints, and classification performances were compared in terms of accuracy, area-under-curve, Matthews correlation coefficient and root mean-squared error. Whereas prediction performance varied with distinct clinical endpoints and classification models, equivalent performance metrics were observed for one- and two-color measurements in both internal and external validation. Furthermore, overlap of selected signature genes correlated inversely with endpoint prediction difficulty. In summary, our data strongly substantiate that the choice of platform is not a primary factor for successful gene-expression based prediction of clinical endpoints.

Translational database iCHIP for the Neuroblastoma Genome Interaction Network

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The sustainable provision of generated resources for the participants across all ENGINE locations is addressed by the proprietary data integration center iCHIP. Initially developed for medical genome research and subsequently tailored towards the needs of Cell Biology and Systems Biology, iCHIP serves as the central data management and data analysis hub for the ENGINE project partners.

More than 10 Neuroblastoma studies including over 1000 experiments are currently stored in iCHIP. The highly flexible framework for comprehensive annotation of experiments integrates necessary specifications for the explorative and integrative analysis of molecular biology result sets. Biological probe annotation and experimental data are integrated and correlated after data curation and congruency checks. The patient and tumor sample information, surveyed by the national NB clinical trial center in Cologne, is uploaded regularly into iCHIP.

iCHIP has been recently enhanced to break down crucial hurdles for the integration of the heterogeneous data types. 1) Typical clinical followup data is related to a probably alternate number of time-points. Time-dependent followup data is now uploaded in a six month time period. This kind of time-point information is directly accessible due to our new applications querying the multi-dimensional structure. 2) The exploitation of all experimental results across data types and different studies is dependent on a comprehensive annotation of relevant molecules. An enhanced version of the annotation system PIMS from the department Molecular Genetics at the DKFZ is now contributing to our gene annotation pipeline. 3) iCHIP has been equiped with an imaging module to include microscopy data from cell-based experiments. ENGINE image data representing the phenotypical behaviour of NB cell lines in response to RNAi knock-down and drug treatment is on the way to be integrated into iCHIP.

A Comparison of Next Generation Sequencing and Microarrays for Transcriptome Expression Profiling

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Microarray based expression profiling has been remarkably successful at elucidating the spatio-temporal patterns of mRNA transcripts within cells and tissues, however there are a number of shortcomings to the existing technology. Both sensitivity and specificity can be low with microarrays. Accuracy can also be negatively affected by the low dynamic range of existing microarray technology. Perhaps more importantly, microarrays restrict the expression profiling data to specific annotations and content that are present. Digital expression profiling using RNAseq and next generation sequencing (NGS) promises to reduce or in some cases eliminate these weaknesses. In order to evaluate the merits of RNAseq for expression profiling, we have performed an extensive comparison of data generated with the ABI SOLiD NGS platform and the Affymetrix U133Plus 2.0 and Human Exon 1.0 ST GeneChip platforms. Using the Microarray Quality Control Consortium RNA control samples as a model system we have demonstrated increased sensitivity, specificity and accuracy of the SoliD RNAseq data relative to the microarray platforms. Both microarray platforms showed similar levels of concordance with the RNAseq data, but the exon arrays provided additional information about exon usage and transcript diversity that were verified by the NGS data. TaqMan PCR was used as a third platform technology to assess relative performance of the NGS and array data and validate the findings for both systems.

Whole-Genome SNP Detection and Haplotype Phasing with Mate Pairs via Ligation-Based Dibase Sequencing

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The HapMap project provides ideal opportunities to fully characterize whole-genome polymorphism events comprising many individuals across multiple populations. Genetic variants, genomic rearrangements and even full-scale de novo sequencing can be characterized rapidly and cost effective. We sequenced a Yoruba HapMap sample, NA18507, using mate-pair libraries (14.9x) with various insert sizes (600bp-3.5kb) as well as fragment libraries (3x). SNP detection was done via a heuristic approach which considers the number of reads per allele as well as a weighted score for each base call. We found 2.33M heterozygous SNPs and 1.53M homozygous SNPs with an overall dbSNP concordance of 81% (dbSNP v129). We evaluated mate pairs which cover 6.18M distinct potential genotypes (our whole genome SNP calls and HapMap genotypes which include reference-allele homozygous loci) and we observed 4.03M potential genotypes covered by 6.77M pairs. Nearly 2/3 of the genotypes are covered by at least 1 mate pair read that is in phase with another genotyped location and 43% that we detect as heterozygous are in phase with another that we also detect as heterozygous. We compared annotated heterozygous HapMap phases to these data and found they are in 98.95% agreement. We also attempted to resolve phases for our 634,568 novel heterozygous SNP calls by interrogating mate pairs pairing to both a novel heterozygous and a HapMap phased heterozygous and found that 76,300 (8.32%) of the novel heterozygous SNPs paired to a HapMap-phased heterozygote. For the 15,946 of these SNPs that have both alleles paired to a HapMap phased heterozygous, the two alleles are in opposite phase as expected 99.52% of the time. The average size of these haplotype blocks is 1.6Kb with blocks extending to as long as 215Kb using multiple mate pairs which overlap with multiple heterozygous loci. We have demonstrated that SOLiD ™ mate-pair data is highly amenable to resolving haplotype phases at single-base pair resolution.

Identification of autoantigenicity patterns in coeliac disease by protein array technology

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Coeliac disease (CD) is a common autoimmune disorder, affecting 0.5-1 % of the Caucasian population. Untreated disease causes tissue destruction of the intestinal mucosa and could increase the risk of establishing other autoimmune diseases. Although a specific diagnostic assay based on the major autoantigen tissue transglutaminase (TG2) exists, new diagnostic antigen markers are necessary to close the gap in diagnostis of CD in IgA deficiency individuals (up to 3% of CD patients). Analysis of autoantigenicity patterns could increase the knowledge about disease manifestation and onset and may also help to find correlations between disease progression and antigen recognition patterns. This might be useful for instance to define the need of therapeutic/dietary intervention.

For screening many proteins in parallel, both protein macroarray and microarray screening technology was applied. In the first instance, macroarrays containing ~38.000 individual clones from the hEx1 expression library (fetal brain cDNA) were screened with CD patient sera. In that screen, 953 individual clones were recorded positive in 55 screens with pooled and individual sera of CD patients. Next, a subset of clones were chosen, expressed and purified and protein microarrays were fabricated. In total, 192 protein microarrays were spotted and incubated with sera of 142 coeliac disease patients and 50 healthy controls and autoantigenicity patterns specific for IgA and IgG antibody-antigen interactions were recorded. Finally, the quality of obtained microarray results was evaluated and the screening results were bioinformatically assessed identifying a number of autoantigens which correlate with coeliac disease and may serve as diagnostic markers for coeliac disease in the future.

National Genome Research Network

Company Satellite Lunch Sessions

Oral presentation abstracts

Illumina UK Ltd.

Illumina's Advances in Next-Generation Sequencing and Genotyping Technologies - Empowering New Science!

Dr. Richard Henfrey, Illumina UK Ltd

Illumina® is committed to providing the scientific community with the latest research tools for Next Generation Sequencing, Genotyping, Custom Genotyping, Gene Expression, Methylation and Cytogenetics. In addition we provide products for clinical applications utilizing our BeadExpress® and VeraCode® technologies. All our research tools are developed on scalable platforms with the increasing demand for more applications in mind.
Recent advances in novel content discovery and scaling of our Beadchip and Sequencing technologies have enabled us to update our Genotyping products and increase the application range for genetic studies from common to rare SNP and CNV discoveries. We will present an update on the latest Genotyping and Sequencing products, and in addition give you an insight into future applications and areas of development for the two platforms.

Roche Applied Science

A quantum leap in high throughput sequencing and real-time-PCR: the Genome Sequencer FLX and LightCycler® 1536 systems

The Roche LightCycler® 1536 Real-Time PCR System - High Throughput Redefined

Dr. Dierk Evers, Roche Diagnostics GmbH, Mannheim

Large-scale gene analyses based on PCR need highly reproducible signal generation, detection and analysis. Temperature control, optical detection system and algorithms for gene characterization need to be highly precise and reliable.
The new Roche Applied Science LightCycler® 1536 meets those demands.

Especially designed for gene expression and gene variation analysis, it generates 1536 data points in less than 60 minutes. This breakthrough to ultra-high plate-based PCR was made possible by a new, cutting-edge plate design. Fast tracking and processing of real-time PCR results is based on an innovative new software concept. The software also offers interfaces for easy data workflow integration. Next generation reagents are specially designed for high throughput and small reaction volumes and enable use of a novel pipetting error-tracking concept

The Genome Sequencer FLX System from Roche - New products and developments

Dr. Guido Kopal, Roche Diagnostics GmbH, Penzberg

The Genome Sequencer FLX System (GS FLX) is a next-generation DNA sequencing technology featuring a unique mix of long reads, exceptional accuracy, and ultra-high throughput. This very versatile next-generation sequencing technology, supports many high profile studies in many application categories. GS FLX users have pursued innovative research in *de novo* sequencing, re-sequencing of whole genomes and target DNA regions, metagenomics, RNA and trancriptome analysis. 454 Sequencing is a powerful tool for human genetics research like re-sequencing the genome of an individual human, the complete human exome and targeted genomic regions using the NimbleGen sequence capture process, detection of low frequency somatic mutations linked to cancer and analysis of genomic structural variations. Also sequencing of highly polymorphic regions like HIV genomes and the human HLA locus were addressed by ultradeep amplicon sequencing.

This presentation will provide a overview about the 454 Sequencing technology, and will focus on the latest product updates like amplicon sequencing for Titanium, sequence capture and new features for transcriptome sequencing.

Applied Biosystems Europe

Accuracy and Throughput of SOLiD™ 3 Plus System Enhances Detection of Variation in Whole Genome and Targeted Resequencing

Raimo Tanzi, Director, Business Development Next Generation Sequencing, Applied Biosystems Europe

Detection of rare variants in the study of genetic diseases and cancer is a fundamental requirement that has not yet been achieved by SNP genotyping methods like GWAS. The introduction of solutions for targeted resequencing of customized sets of genes, or for whole exome analysis, combined with growing throughput and declining cost per gigabase sequenced, now make it possible to detect variation in many samples at a reasonable cost level. The SOLiD™ 3 Plus System, paired with Agilent or febit enrichment solutions, offers new possibilities to achieve the highest accuracy and ensure a higher percentage of detection of variants at any given coverage.

Febit Biomed GmbH

The Power to Detect

Dr. Nadine Schracke, Febit Biomed GmbH

Genomics research has been boosted into a new dimension through the immense output capability of next generation sequencers. With a sequencing output of several gigabase-pairs per week with a single benchtop instrument, many large scale experiments have become feasible at reasonable costs. However, to fully make use of this sequencing capability, new protocols and methodologies had to be developed for pre-sequencing sample preparation, which still presents as a bottle-neck compared to the mere sequencing step. Especially targeted enrichment of selected genomic subsets has proven to be a bottle-neck and robust methods are still lacking. In addition, there is a strong need to increase sample size (e.g. for targeted resequencing of large case-control collections) through barcoding, i.e. multiplexing strategies. HybSelect is a solution for this bottle-neck. It is a microarray-based sequence capture method with very efficient enrichment and resequencing performance. We present here various successful enrichment studies including our first results with multiplexing.

Affymetrix Europe

Conquer the next wave of genomic discoveries: the next-generation Axiom™ Genotyping Solution from Affymetrix®

Dr. Dirk Jürgensen, Affymetrix Europe

Study of complex disease genetics is moving into an exciting new era as rare variants and other classes of polymorphism offer new opportunities. Affymetrix has launched the next-generation Axiom Genotyping Solution to equip the research community as it conquers the next wave of genomic discoveries.
Axiom enables access to new genomic content such as the output of the 1000 Genomes Project; it provides flexible array content to focus on specific populations, genomic regions or types of variants; it delivers reduced cost per sample and high-throughput to power next-generation genetics studies; it offers a single platform for seamless transition from GWAS through follow-up studies for fine-mapping and validation. The Axiom™ Genotyping Solution is available on the Affymetrix® GeneTitan™ instrument platform, the world's first hands-free microarray system.
Join us for our Satellite Lunch Session to learn how the next-generation Axiom Genotyping Solution can provide you with flexible content, fast results, more power and greater confidence to conquer the next wave of genomic discoveries.

Fluidigm Europe B.V.

Library preparation for re-sequencing and long range sequencing made easy and robust using Fluidigm's proven nanofluidic technology

Harry Boeltz, Fluidigm Europe B.V., Netherlands

Radically simplified library preparation and massively enabled multiplexed sequencing.
One of the largest challenges facing next-generation sequencing operators today is how to utilize the massive amounts of throughput enabled by the new crop of instruments. While all of the systems in use today allow massive amounts of data to be generated on a per sample basis, they lack a simple and reliable method of running multiple samples per run, and thus harvesting the tremendous throughput of these instruments.
The Fluidigm Access Array™ IFC eases the burden of library preparation because it facilitates the amplification and barcoding of 48 samples in parallel, with minimal time and labor and in as few as three hours.

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Zimmermann	Karin		Humboldt-Universität zu Berlin	karin.zimmermann@informatik.hu-berlin.de
Zimmermann	Wolfgang	Dr.	AGOWA GmbH	zimmermann@agowa.com
Zollbrecht	Christa		Universitätsklinikum Regensburg	christa.zollbrecht@klinik.uni-regensburg.de
Zouhair	Aherrahrou	PhD	Universität zu Lübeck	zouhair.aherrahrou@uk-sh.de
Zweier	Markus		University of Erlangen-Nuremberg	mzweier@humgenet.uni-erlangen.de

National Genome Research Network

List of NGFN-Plus Integrated Genome Research Networks and NGFN-Transfer Innovation Alliances

IG Atherogenomics

Koordination: Prof. Dr. Heribert Schunkert

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Schunkert	Heribert	Prof. Dr. med.	Universität zu Lübeck	A1a, A2a, E1, F1	**A. Explorative Genomics**
Erdmann	Jeanette	Prof. Dr. rer. nat.	Universität zu Lübeck	A1a, A2a C2, F1	A1 Polygenic and monogenic forms of MI
Linsel-Nitschke	Patrick	Dr. med.	Universität zu Lübeck	A2a, D1, D2a	A2 Genomics of coronary artery disease
Aherrarhou	Zouhair	Dr. med.	Universität zu Lübeck	B2	A3 Genomics of sub clinical atherosclerosis
Ehlers	Eva-Maria	PD Dr. med.	Universität zu Lübeck	B2	**B. Comparative Genomics**
Döhring	Lars	Dr. med.	Universität zu Lübeck	B2	B1 Syntenic regions for atherosclerosis in mice and humans
Fischer	Marcus	PD. Dr. med.	Universität zu Regensburg	A2b, D2b	B2 ABCC6 and arterial calcification
Hengstenberg	Christian	Prof. Dr. med.	Universität zu Regensburg	A1b, C1, E1	**C. Population Genetics**
Teupser	Daniel	PD Dr. med.	Universität Leipzig	B1	C1 Cases and population platform (KORA/MONICA; GMIS;PREVENT-IT, LE HEART)
Thiery	Joachim	Prof. Dr. med.	Universität Leipzig	B1	C2 Genetic epidemiology methods platform
Blankenberg	Stefan	Prof. Dr. med.	Klinikum der Johannes Gutenberg-Universität	A3a, D1, E1	**D. Functional Genomics**
Zeller	Tanja	Dr. rer. nat.	Klinikum der Johannes Gutenberg-Universität	A3a, D1, E1	
Steller	Ulf	Dr. rer. nat.	Euroimmun AG	E1	D1 Gene expression profiling Transcriptome of monocytes in subclinical atherosclerosis and MI patients
Koenig	Wolfgang	Prof. Dr. med.	Universitätsklinikum Ulm	A3b, C1, E2	D2 Genomics of plasma lipids
König	Inke	PD Dr. rer. nat.	Universität zu Lübeck	C2b	**E. Transfer**
Wichmann	Erich	Prof. Dr. rer. nat. Dr. med.	Helmholtz Zentrum München	C1	E1 SNP array for atherosclerosis Development of innovative diagnostics
Ziegler	Andreas	Prof. Dr. rer. nat.	Universität zu Lübeck	C2b	E2 50 K Vascular Disease SNP Array
Meitinger	Thomas	Prof. Dr. med.	Helmholtz Zentrum München	CF	**F. Organisation**
					F1 Coordinating office
					Genotyping facility
					CF Genotyping/sequencing facility

IG Genetics of Heart Failure (Genetik der Herzinsuffizienz)					
Koordination: Prof. Dr. Hugo A. Katus					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	1a	Genetic Risk of Heart Failure and its Subphenotypes
Pieske	Burkert	Prof. Dr.	Georg-August-Universität Göttingen	1b	Genetic Risk of Heart Failure and its Subphenotypes
Hasenfuß	Gerd	Prof. Dr.	Georg-August-Universität Göttingen	1b	Genetic Risk of Heart Failure and its Subphenotypes
Kääb	Stefan	Prof. Dr.	Ludwig-Maximilians-Universität München	1c	Genetic Risk of Heart Failure and its Subphenotypes
Kreutz	Reinhold	Prof. Dr.	Charite Universitätsmedizin, CBF	2	Systems Biology Genomics of Left Ventricular Hypertrophy (LVH) using congenic rat models of polygenic hypertension
Hübner	Norbert	Prof. Dr.	Max-Delbrück-Centrum für molekulare Medizin	3	Gene Regulatory Networks in Cardiac Hypertrophy and Failure
Ivandic	Boris	Dr.	Universitätsklinikum Heidelberg	4	Genetic Modifiers of Heart Failure in Mice
Rottbauer	Wolfgang	PD Dr.	Universitätsklinikum Heidelberg	5	Functional Genomics in Zebrafish to Dissect the Genetics of Human Myocardial Disease
Frey	Norbert	PD Dr.	Universitätsklinikum Heidelberg	6	Novel Molecular Pathways in Cardiac Hypertrophy and Failure
Knöll	Ralph	Prof. Dr.	Georg-August-Universität Göttingen	7	Genetics and Functional Analysis of Cardiac Mechano sensation - Relevance for the Pathophysiology of Diastolic Heart Failure
Guan	Kaomei	Dr.	Georg-August-Universität Göttingen	7	Genetics and Functional Analysis of Cardiac Mechanosensation - Relevance for the Pathophysiology of Diastolic Heart Failure
Lehnart	Stephan	Dr.	Georg-August-Universität Göttingen	8	Molecular Genomics Intracellular Calcium-Handling in Diastolic Dysfunction, Heart Failure and Arrhythmias
Pieske	Burkert	Prof. Dr.	Georg-August-Universität Göttingen	8	Molecular Genomics Intracellular Calcium-Handling in Diastolic Dysfunction, Heart Failure and Arrhythmias
Weis	Tanja	Dr.	Universitätsklinikum Heidelberg	9	Coordination Office
Stoll	Monika	Prof. Dr.	Leibniz-Institut für Arteriosklerose forschung an der Universität Münster	10	Genetic epidemiology of Heart Failure: Genetic Epidemiological Support for the IG

Eils	Roland	Prof. Dr.	DKFZ	11	Bioinformatic Methods
Brors	Benedikt	Dr.	Deutsches Krebsforschungs-zentrum	11	Bioinformatic Methods
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	12	DNA-Platform
Meder	Benjamin	Dr.	Universitätsklinikum Heidelberg	12	DNA-Platform
Rottbauer	Wolfgang	PD Dr.	Universitätsklinikum Heidelberg	13	High-throughput functional in vivo evaluation of heart failure associated genes and pathways by Morpholino knock-down in zebrafish

IG Molekulare Mechanismen der Adipositas

Koordination: Prof. Dr. Johannes Hebebrand

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Hinney	Anke	PD Dr.	Universität Duisburg-Essen	TP1	Identification of human obesity genes with a focus on developmental aspects
Schürmann	Annette	Prof. Dr.	Deutsches Institut für Ernährungs-forschung (DIfE)	TP2	Identification and characterization of obesity genes, gene-gene and diet gene interactions involved in polygenic obesity in mice
Klingenspor	Martin	Prof. Dr.	Technische Universität München	TP3a	Alterations of the mouse brain proteome associated with the early development of diet-induced obesity in the mouse
Stühler	Kai	Dr.	Ruhr-Universität Bochum	TP3b	Alterations of the mouse brain proteome associated with the early development of diet-induced obesity in the mouse
Illig	Thomas	PD Dr.	Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	WB2-Aa	Evaluation of candidate genes for obesity and related disorders in large representative epidemiological cohorts encompassing children and adults - KORA
Reinehr	Thomas	PD Dr.	Institut für Pädiatrische Ernährungsmedizin, Vestische Kinder- und Jugendklinik, Universität Witten/Herdecke	WB2-Ab	Evaluation of candidate genes for obesity and related disorders in large representative epidemiological cohorts encompassing children and adults - Obeldicks
Krude	Heiko	Prof. Dr.	Charité	WB2-B	WB2-BEPOC
Moebus	Susanne	PD Dr.	Universität Duisburg-Essen	WB2-C	WB2-RECALL
Wabitsch	Martin	Prof. Dr.	Universität Ulm	WB2-D	WB2-UPOC
Rosskopf	Dieter	Prof. Dr.	Universität Greifswald	WB2-E	WB2-SHIP
Boeing	H.	Prof. Dr.	Deutsches Institut für Ernährungs-forschung (DIfE)	WB2-F	WB2-EPIC

Klingenspor	Martin	Prof.Dr.	Technische Universität München	WB3-Aa	Novel mouse models for the evaluation of candidate genes in the physiology of energy balance regulation
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	WB3-Ab	Novel mouse models for the evaluation of candidate genes in the physiology of energy balance regulation
Fischer-Posovszky	Pamela	Dr.	Universität Ulm	WB3-B	Adipogenese
Horsthemke	Bernhard	Prof. Dr.	Universität Duisburg-Essen	WB3-C	Allelische Expression
Biebermann	Heike	Dr.	Charité	WB3-C	Methylierung
Brockmann	Gudrun	Prof. Dr.	Humboldt-Universität zu Berlin	WB3-D	Bioinformatik
Rüther	Ulrich	Prof. Dr.	Heinrich-Heine-Universität Düsseldorf	TP10	Investigation of Fto as a major contributor to obesity
Sauer	Sascha	Dr.	Max-Planck-Institut für Molekulare Genetik (MPIMG)	TP11a	Systematic molecular characterisation of compounds for prevention and therapy of obesity and insulin resistance
Büssow	Konrad	Dr	HZI Braunschweig	TP11b	Systematic molecular characterisation of compounds for prevention and therapy of obesity and insulin resistance
Blüher	Matthias	Prof. Dr.	Universität Leipzig	TP12	Adverse effects of weight cycling on longevity in rodents
Brockmann	Gudrun	Prof. Dr.	Humboldt-Universität zu Berlin	TP14	Implications of diet and exercise with interaction of allelic variations in the Berlin Fat Mouse line
Schäfer	Helmut	Prof. Dr.	Philipps-Universität Marburg	TP15a	Central statistical genomics and data management
Scherag	André	Dr.	Universität Duisburg-Essen	TP15b	Central statistical genomics and data management
Hebebrand	Johannes	Prof. Dr.	Universität Duisburg-Essen	TP16	Coordination and quality management

IG Pathogenic role of mi-RNA in Herpes Infections

Koordination: Prof. Dr. Dr. Jürgen G. Haas

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Haas	Jürgen G.	Prof. Dr. Dr.	LMU , München	1	Herpesviral factors modulating the cellular miRNA processing machinery
Koszinowski	Ulrich	Prof. Dr.	LMU	2	Characterization of CMV miRNAs in vitro and in vivo
Adler	Heiko	PD Dr.	Helmholtz-Zentrum	3	In vivo effects of miRNAs inthe murine herpesvirus 68 (mHV-68)
Grässer	Friedrich	Prof. Dr.	Universitätsklinik des Saarlandes	4	Function of EBV-encoded and EBV-induced mirRNA in latency and transformation

Meister	Gunther	Dr.	Max-Planck Institut	5	Identification of cellular targets of viral miRNAs
Förstemann	Klaus	Prof. Dr.	LMU	6	Biochemical interaction of viral and cellular miRNAs
Zimmer	Ralf	Prof. Dr.	LMU	7	Prediction of viral miRNAs targets

IG RNomics in Infections

Koordination: Prof. Dr. Jürgen Brosius

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Reinhardt	Richard	Dr.	MPI für Molekulare. Genetik	1	Ultra-High-Parallel Sequencing and Biocomputational Analysis of npcRNA
Vogel	Jörg	Dr.	MPI für Infektionsbiologie	2	RNomics of bacterial infections
Rudel	Thomas	Prof.	Universität Würzburg	2	RNomics of bacterial infections
Walter	Lutz	PD Dr.	Deutsches Primatenzentrum Göttingen	3	RNomics of viral infections
Brosius	Jürgen	Prof. Dr.	Universität Münster	4	RNomics of eukaryotic parasites

IG Systematic Genomics of Chronic Inflammatory Barrier Diseases

Koordination: Prof. Dr. Stefan Schreiber

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Schreiber	Stefan	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	T7	Koordination
Franke	Andre	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 1	Genetische Ätiologie des M. Crohn
Rüther	Andreas	Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 5	Genetische Ätiologie der atopischen Dermatitis
Fölster-Holst	Regina	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 5	Genetische Ätiologie der atopischen Dermatitis
Nebel	Almut	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 6	Genetische Ätiologie der Psoriasis
Weichenthal	Michael	PD. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 6	Genetische Ätiologie der Psoriasis
Nikolaus	Susanna	PD Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 7	Genetische Ätiologie der Colitis ulcerosa
Schreiber	Stefan	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 8	Follow up Genotypisierung i.d. Teilprojekten GP 1, 2, 4-7

Till	Andreas	Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 9	Funktionelle Aufklärung
Platzer	Matthias	PD Dr.	FLI- Leibniz-Institut für Altersforschung	GP 9	Funktionelle Aufklärung
Rosenstiel	Philip	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	GP 10	Systematische Aufklärung von Signaltransduktionswegen: angeborene Immunität
Hofmann	Sylvia	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	T1	Qualitätsmanagenment
Wittig	Michael	ohne	UNIKLINIK Schleswig-Holstein , Campus Kiel	T3a	Bioinformatische Unterstützung
Schilhabel	Markus	Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	T1	Quätlitätsmanagenment
Jacobs	Gunnar	Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	T5a	Hochdurchsatz zelluläre Screening Assays via Rna Interferenz
Krawczak	Michael	Prof. Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	T2	Genetisch-epidemiologische Unterstützung
Nothnagel	Reiner	Dr.	UNIKLINIK Schleswig-Holstein , Campus Kiel	T2	Genetisch-epidemiologische Unterstützung
Kabesch	Michael	Prof. Dr.	Medizinische Hochschule Hannover	GP 2	Genetische Ätiologie des Asthma bronchiale
Horstmann,	Rolf	Prof.	Bernhard-Nocht-Institut für Topenmedizin	GP 4	Genetischer Ätiologie der Tuberkulose
Meyer	Christian	Prof. Dr.	Bernhard-Nocht-Institut für Topenmedizin	GP 4	Genetischer Ätiologie der Tuberkulose
Lee	Young-Ae	Prof. Dr.	Charité, Campus Virchow-Klinikum	GP 5	Genetische Ätiologie der atopischen Dermatitis
Vingron	Martin	Prof. Dr.	Max Planck Institut für Molekulare Genetik (MPI-MG)	T 3b	Bioinformatische Unterstützung
Albrecht	Mario	Dr.	Max Planck Institut für Informatik (MPI-INF)	T 3c	Bioinformatische Unterstützung
Weidinger	Stefan	PD Dr.	Technische Universität München, Klinikum Rechts der Isar	GP 5	Genetische Ätiologie der atopischen Dermatitis
Kaufman	Stefan H.E.	Prof. Dr.	Max Planck Institut für Infektions-biologie	GP11	Systematische Aufklärung von Stoffwechelwegen: Adaptive Immunität
Wiemann	Stefan	PD Dr.	Deutsches Krebsforschungs-zentrum - DKFZ	T5b	Hochdurchsatz zelluläre Screening Assays via RNA Interferenz

IG Functional and Translational Genomics of Acute Leukemias

Koordination: Prof. Dr. Christian Hagemeier

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Hubert	Serve	Prof. Dr.	Uni Frankfurt	TP3a	
Hagemeier	Christian	Prof. Dr.	Charité	TP14, TP16	
Ludwig				TP13	
Döhner	Hartmut	Prof. Dr.	Uni Ulm	TP1	
Thiede	Christian	Prof. Dr.	TU Dresden	TP2	
Müller-Tidow	Carsten	Prof. Dr.	Uni Münster	TP3b	
Kulozik	Andreas	Prof. Dr.	Uni Heidelberg	TP4	
Marschalek	Rolf	Prof. Dr.	Uni Frankfurt	TP5	
Bohlander	Stefan	Prof. Dr.	LMU München	TP6	
Feuring-Buske	Michaela	PD. Dr.	Helmholtz - Zentrum München	TP7	
Leutz	Achim	Prof. Dr.	MDC Berlin	TP8	
Duyster	Justus	Prof. Dr.	TU München	TP9	
Grez	Manuel	Prof. Dr.	GSH Frankfurt	TP10	
Neubauer	Andreas	Prof. Dr.	Uni Marburg	TP11	
Schrappe	Martin	Prof. Dr.	Uni Kiel	TP12	
Lottaz	Claudio	Dr.	Uni Regensburg	TP15	(Weiterer TP-Leiter: Prof. Dr. R. Spang)

IG Brain Tumor Network

Koordination: Prof. Dr. Peter Lichter

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Lichter	Peter	Prof. Dr.	DKFZ	SP-C	Koordinierung und Lenkung des Netzwerkes BTN^{plus}
Lichter	Peter	Prof. Dr.	DKFZ	SP-1	Hochdurchsatzanalyse von potentiellen Onkogenen und Tumorsuppressorgenen in Gliomen
Wolter	Marietta	Dr.	Heinrich-Heine-Universität	SP-2a	Aberrante miRNA-Expression in Gliomen: Molekulare Mechanismen, funktionelle Konsequenzen und deren klinische Signifikanz
Stühler	Kai	Dr.	Ruhr-Universität Bochum	SP-2b	Aberrante miRNA-Expression in Gliomen: Molekulare Mechanismen, funktionelle Konsequenzen und deren klinische Signifikanz
Brors	Benedikt	Dr.	DKFZ	SP-3	Modellierung und Bioinformatik
Hahn	Meinhard	Dr.	Universitätsklinikum Hamburg-Eppendorf	SP-4	Funktionelle Charakterisierung der an Hypoxie und Sauerstoff-metabolismus beteiligten Gene *Cited4* und *PRDX1*, die günstiges Therapieansprechverhalten und verbessertes Gesamtüberleben bei Gliompatienten vorhersagen

Acker	Till	Prof. Dr.	Universitätsklinikum Gießen und Marburg GmbH	SP-5	Selbsterneuerungs- und Differenzierungsmechanismen in Gliom-Stammzellen
Wick	Wolfgang	Prof. Dr.	DKFZ	SP-6a	Funktionelle Charakterisierung durch chronische nicht-lethale Hypoxie induzierter Invasions-assoziierter Proteine
Vajkoczy	Peter	Prof. Dr.	Charité - Medizinische Universität Berlin	SP-6b	Validierung hypoxie-regulierter Moleküle für Tumorinvasion und Angiogenese
Hau	Peter	Dr.	Universität Regensburg	SP-7	Dysregulierte Migration und Differenzierung - molekulare und zelluläre Dissektion von Krebs-stammzellen in hochgradigen Gliomen
Waha	Andreas	Dr.	Universitätsklinikum Bonn	SP-8	Funktionelle Bedeutung epigenetisch deregulierter Gene in Gliomen
Angel	Peter	Prof. Dr.	DKFZ	SP-9a	Funktionelle Analyse der KLK-ADAM-Achse bei der Zellmigration und Invasion von humanen Gliomen
Pietsch	Torsten	Prof. Dr.	Universitätsklinikum Bonn	SP-9b	Funktionelle Analyse der KLK-ADAM-Achse in der Migration und Invasion von Glioblastomen
Roth	Wilfried	Dr.	DKFZ	SP-10	Neue Funktionen von BCL2-Familien-Proteinen: Invasivität und Autophagie
Reifenberger	Guido	Prof. Dr.	Heinrich-Heine-Universität	SP-11a	Molekulare und funktionelle Charakterisierung von Genen, welche die Zellteilungssymmetrie in malignen Gliomen kontrollieren
Radlwimmer	Bernhard	Dr.	DKFZ	SP-11b	Molekulare und funktionelle Charakterisierung von Genen, welche die Zellteilungssymmetrie in malignen Gliomen kontrollieren
Herold-Mende	Christel	PD Dr.	Universität Heidelberg	SP-12a	Funktionelle Analysen von differenzierungsrelevanten Kandidatengenen in Gliomstammzellen
Radlwimmer	Bernhard	Dr.	DKFZ	SP-12b	Funktionelle Analysen von differenzierungsrelevanten Kandidatengenen in Gliom-Stammzellen
Hartmann	Christian	PD Dr.	DKFZ	SP-13	Funktionelle Charakterisierung der putativen Tumorsuppressorgene *EMP3* und *ST13* in Gliomen
Wick	Wolfgang	Prof. Dr.	Universität Heidelberg	SP-14	Klonierung und funktionelle Charakterisierung des murinen Regenerations- und Toleranz-Faktors: ein Glioma-Autoantigen-Kandidat mit immuno-suppressiven Eigenschaften

IG Integrated Genome Network of Prostate Cancer Koordination: PD Dr. Holger Sültmann					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Schlomm	Thorsten	Dr.	Martini-Klinik Prostatakrebszentrum und UKE Hamburg	TP1	Kollektivierung und Bereitstellung von klinischen Proben und Patientendaten
Simon	Ronald	PD Dr.	UKE Hamburg-Eppendorf	TP2	Biologische und klinische Signifikanz von Mikro-amplifikationen im Prostatakarzinom
Dierlamm	Judith	Dr.	UKE Hamburg-Eppendorf	TP3	Zytogenetische und molekulare Charakterisierung von Translokations-Bruchpunkten im Prostatakarzinom
Yekebas	Emre	Prof. Dr.	UKE Hamburg-Eppendorf	TP3	Zytogenetische und molekulare Charakterisierung von Translokations-Bruchpunkten im Prostatakarzinom
Schweiger	Michal-Ruth	Dr.	MPI für Molekulare Genetik	TP4	Analyse von Mutationen und epigenetischen Veränderungen im Prostatakarzinom
Lehrach	Hans	Prof. Dr.	MPI für Molekulare Genetik	TP4	Analyse von Mutationen und epigenetischen Veränderungen im Prostatakarzinom
Sültmann	Holger	PD Dr.	Deutsches Krebsforschungs-zentrum	TP5	Splice-Varianten- und miRNA Expression in Tumoren
Brümmen-dorf	Tim	Prof. Dr.	Universitätsklinikum Aachen	TP6	Identifizierung klinisch relevanter Proteine im Prostatakarzinom
Heitmann	Alke	Dr.	Qiagen Hamburg GmbH	TP7	Entwicklung und Kommerzialisierung eines diagnostisch einsetzbaren Tools zur Detektion molekularer Marker im Prostatakarzinom
Haese	Alexander	PD Dr.	Martini-Klinik Prostatakrebszentrum und UKE Hamburg	TP8	Identifizierung und Validierung von diagnostischen und prognostischen Markern für die Therapieentscheidung beim Prostatakarzinom
Korf	Ulrike	Dr.	Deutsches Krebsforschungs-zentrum	TP9	Proteinarrays zur quantitativen Analyse von Proteinen in Tumoren und in Patientenseren
Weller	Horst	Prof. Dr.	Centrum für Angewandte Nanotechnologie (CAN) GmbH	TP10	Molekulare Tumor-Bildgebung mit Hilfe Antikörper-gekoppelter Nanopartikel
Mollenhauer	Jan	Prof. Dr.	Deutsches Krebsforschungs-zentrum	TP11	Funktionelle zelluläre Assays in Prostatakarzinom-Zelllinien
Sültmann	Holger	PD Dr.	Deutsches Krebsforschungs-zentrum	TP12	In vivo Analyse von Genen im Prostatakarzinom

Beissbarth	Tim	Dr.	Deutsches Krebsforschungs-zentrum	TP13	Bioinformatik und Systembiologie
Sültmann	Holger	PD Dr.	Deutsches Krebsforschungs-zentrum	TP14	Koordinierung, Kommunikation und Qualitätsmanagement
IG ENGINE (**E**xtended **N**euroblastoma **G**enome **I**nteraction **NE**twork)					
Koordination: Prof. Dr. Angelika Eggert					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Berthold	Frank	Prof. Dr.	Klinikum der Universität zu Köln, Zentrum für Kinder-heilkunde und Jugendmedizin	2a	Central database & tumorbank
				8	Predictive gene signatures and transcription regulatory networks
Brors	Benedikt	Dr.	Deutsches Krebsforschungszentrum	7	Biostatistics for molecular trial design
Deubzer	Hedwig	Dr.	Deutsches Krebsforschungs-zentrum	3	Identification of NB initiating cells
				14	Targeting class I histone deacetylases
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	1	Project management
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	4a	Proteomics of NB master regulators
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	9a	NB Toponome
Eilers	Martin	Prof. Dr.	Philipps-Universität Marburg	11	Systematic drug testing
Fischer	Matthias	PD Dr.	Klinikum der Universität zu Köln, Zentrum für Kinderheilkunde und Jugendmedizin	8	Predictive gene signatures and transcription regulatory networks
Ivics	Zoltan	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	5a	Identification of NB initiating genes
König	Rainer	Dr.	Institut für Pharmazie und Molekulare Biotechnologie/Bioquant	12	Refined treatmend selection with machine learning techniques
Lawerenz	Christian		Deutsches Krebsforschungs-zentrum	2b	Central database & tumorbank

Lode	Holger	Prof. Dr.	Charité Campus Virchow-Klinikum	15	Genetic vaccination
Oberthür	André	Dr.	Zentrum für Kinderheilkunde	9b	NB Toponome
Savelyeva	Larissa	Dr.	Deutsches Krebsforschungs-zentrum	10	NB Fragilome
Schramm	Alexander	Dr.	Universitäts-Kinderklinik Essen	4a	Proteomics of NB master regulators
				6	Role of microRNAs in NB pathogenesis
Schubert	Walter	Dr.	Otto-von-Guericke-Universität Magdeburg	9c	NB Toponome
Schulte	Johannes H.	Dr.	Universitäts-Kinderklinik Essen	5b	Identification of NB initiating genes
				6	Role of microRNAs in NB pathogenesis
Schwab	Manfred	Prof. Dr.	Deutsches Krebsforschungszentrum	10	NB Fragilome
				13	Targeting Myc functions
Stühler	Kai	Dr.	Ruhr-Universität Bochum	4b	Proteomics of NB master regulators
Westermann	Frank	Dr.	DKFZ	13	Targeting Myc functions
Witt	Olaf	Prof. Dr.	Deutsches Krebsforschungs-zentrum	3	Identification of NB initiating cells
				14	Targeting class I histone deacetylases

IG Deciphering Oncogene Dependencies in Human Cancer Oncogene Mutation Space

Koordination: Dr. Roman Thomas

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Thomas	Roman	Dr.	Max-Planck-Institute	0	Coordinating office
Nürnberg	Peter	Prof. Dr.	University of Cologne	1	Evaluation of tools for clinical detection of mutations and copy number changes
Wolf	Jürgen	Prof. Dr.	University Clinic Cologne	2	Analysis of patient mutation space and clinical outcome
Thomas	Roman	Dr.	Max-Planck-Institute	3	Systematic high-throughput analysis of oncogenicity of human oncogene mutations
Ahmadian	Reza	PD Dr.	Heinrich-Heine Universtity Hospital	4a	Functional impact of oncogene mutants and small molecules on the Ras and Rho signaling pathways
Wittinghofer	Alfred	Prof. Dr.	MPI für molekulare Physiologie	4b	Functional impact of oncogene mutants and small molecules on the Ras and Rho signaling pathways
Rauh	Daniel	Dr.	Max Planck Society	5a	Dissection of oncogene dependencies by small organic molecule perturbations

Waldmann	Herbert	Prof. Dr.	Max Planck Institute	5b	Dissection of oncogene dependencies by small organic molecule perturbations
Rahnenführer	Jörg	Prof. Dr.	University Dortmund	6b	Statistical modeling of drug response and pathway alterations
Lengauer	Thomas	Prof. Dr. Dr.	MPI für Informatik	6a	Statistical modeling of drug response and pathway alterations
IG Systems Biology of Genetic Diseases, Mutanom					
Koordination: Prof. Dr. Hans Lehrach					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Schweiger	Michal	Dr.	Max-Planck-Institut für Molekulare Genetik	TP3	Mutational analysis
Mollenhauer	Jan	Prof. Dr.	Medical Bio-technology Center University of Southern Denmark	TP4	Recombinant cancer cell libraries & drug target recovery
Sültmann	Holger	PD Dr.	German Cancer Research Center	TP5	Quantification of cancer pathways
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	TP6	Protein interaction networks
Schultze-Motel	Paul	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	TP6	Protein interaction networks
Schäfer	Reinhold	Prof.	Charité Universitätsmedizin Berlin	TP7	Cellular signalling networks
Herrmann	Bernhard	Prof.	Max-Planck-Institut für Molekulare Genetik	TP8	Mouse disease models
Morkel	Markus	Dr.	Max-Planck-Institut für Molekulare Genetik	TP8	Mouse disease models
Lange	Bodo	PD Dr.	Max-Planck-Institut für Molekulare Genetik	TP9	Protein complex composition and function in disease
Wierling	Christoph		Max-Planck-Institut für Molekulare Genetik	TP10	Data integration and modelling
Drewes	Gerard	Dr.	Cellzome AG	TP11	Quantitative Proteomics
Joberty	Gerard	Dr.	Cellzome AG	TP11	Quantitative Proteomics
IG Translational Genome Research Network in Pancreatic Cancer Koordination: Prof. Dr. Thomas M. Gress					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Gress	Thomas M.	Prof. Dr.	Philipps-Universität Marburg	TP0,TP1b, TP2b,	TP0 Koordination, TP1b Klinische Ressourcen und Daten, TP2b

					Mausmodelle des Pankreas-karzinoms
Buchholz	Esther		Philipps-Universität Marburg	TP 0	TP 0 Koordination
Tannapfel	Andrea	Prof. Dr.	Ruhr-Universität Bochum	TP1c	Klinische Ressourcen und Daten
Giese	Nathalia	Dr.	Universitätsklinikum Heidelberg	TP1a	Klinische Ressourcen und Daten
Sipos	Bence	Prof. Dr.	Eberhard-Karls Universität Tübingen	TP1d	Klinische Ressourcen und Daten
Sipos	Bence	Prof. Dr.	Eberhard-Karls Universität Tübingen	TP2c	Mausmodelle des Pankreaskarzinoms
Schmid	Roland M.	Prof. Dr.	TU München	TP2a	Mausmodelle des Pankreaskarzinoms
Buchholz	Malte	PD Dr.	Philipps-Universität Marburg	TP3, TP11a	TP3 Parallelisierte funktionelle Charakterisierung, TP11 Molekulare Differentialdiagnose
Seufferlein	Thomas	Prof. Dr.	Martin-Luther-Universität Halle-Wittenberg	TP4	Kinasenetzwerke im Pankreaskarzinom
Kestler	Hans	Prof. Dr.	Uniklinik Ulm	TP11b	Molekulare Differentialdiagnose
Hoheisel	Jörg	Dr.	DKFZ Heidelberg	TP5, TP12	TP5 Quantitative Analyse von Proteininteraktionen, TP12 Epi-genetische Analyse zur therapeu-tischen Patienten-Stratifizierung
Hahn	Stephan	Prof. Dr.	Ruhr-Universität Bochum	TP6	MiRNAs als therapeutische Targets für das Pankreaskarzinom
Friess	Helmut	Prof. Dr.	TU München	TP7	Molekulare Analyse der tumorspezifischen Stromaaktivierung
Kleeff	Jörg	PD Dr.	TU München	TP7	Molekulare Analyse der tumorspezifischen Stromaaktivierung
Schwarte-Waldhoff	Irmgard	PD Dr.	Ruhr-Universität Bochum	TP9a	Entwicklung von molekular-diagnostischen Verfahren zur Früherkennung des Pankreas-karzinoms basierend auf sezernierten/freigesetzten Kandidaten-Proteinen
Schnölzer	Martina	Dr.	DKFZ Heidelberg	TP9b	Entwicklung von molekular-diagnostischen Verfahren zur Früherkennung des Pankreas-karzinoms basierend auf sezernierten/freigesetzten Kandidaten-Proteinen
Günther	Simone	Dr.	Applied Biosystems	TP11c	Molekulare Differentialdiagnose

IG Modifiers of Intestinal Tumor Formation and Progression

Koordination: Prof. Dr. Bernhard Herrmann

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Schweiger	Michal	Dr.	Max-Planck-Institut für molekulare Genetik	1	Analyse von normalem und Darmtumorgewebe und Validierungsexperimente in

					menschlichen Zelllinien
Herrmann	Bernhard	Prof.Dr.	Max-Planck-Institut für molekulare Genetik	2	Identifizierung und Feinkartierung von Modulatoren der epi-genetischen Genkontrolle und APC-Min induzierter Darmtumore in CSS Mausstämmen
Lehrach	Hans	Prof. Dr	Max-Planck-Institut für molekulare Genetik	3	Immunpräzipitation von methylierter DNA und Gen-Expressionsanalyse mittels der Sequenziertechnik der 2. Generation
Walter	Jörn	Prof.Dr.	Universität des Saarlandes, Campus Saarbrücken	4	Entwicklung einer Bisulphit-Hochdurchsatz-Sequenzierungs-platform in Kombination mit integrierter Bioinformatik
Morkel	Markus	Dr.	Max-Planck-Institut für molekulare Genetik	5	Validierung von Kandidatengenen (Modifier) in transgenen Mausmodellen
Herwig	Ralf	Dr.	Max-Planck-Institut für molekulare Genetik	6	Bioinformatik und Datenintegration

IG Integrated Genomic Investigation of Colorectal Carcinoma (CRC)

Koordination: Prof. Dr. Kari Hemminki

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Hampe	Jochen	PD Dr.	Universitätsklinikum Schleswig-Holstein	TP1	Fine mapping + replication
Hemminki	Kari	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based studies
Schafmayer	Clemens	Dr.	Universitätsklinikum Schleswig-Holstein	TP2	Population-based and prospective validation
Chang-Claude	Jenny	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based and prospective validation
Brenner	Hermann	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based and prospective validation
Burwinkel	Barbara	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based and prospective validation
Krawczak	Michael	Prof. Dr.	Universitätsklinikum Schleswig-Holstein	TP3	Statistics and Genetic epidemiology
Brosch	Mario	Dr.	Universitätsklinikum Schleswig-Holstein	TP4	Somatic mutation signature
Platzer	Matthias	Prof. Dr.	Leibniz-Institut für Altersforschung	TP4	Somatic mutation signature

Siebert	Reiner	Prof. Dr.	Universitätsklinikum Schleswig-Holstein	TP5	Somatic genomic imbalances, LOH and methylation
Boutros	Michael	Prof. Dr.	Deutsches Krebsforschungszentrum	TP6	Pathways - cellular models
Spang	Rainer	Prof. Dr.	Universität Regensburg	TP7	System biology of the cancer cell
Kalthoff	Holger	Prof. Dr.	Universitätsklinikum Schleswig-Holstein	TP8	Pathways: tumor tissue
Hemminki	Kari	Prof. Dr.	DKFZ	TP9	Coordination

IG MooDS: Systematic Investigation of the Molecular Causes of Major Mood Disorders and Schizophrenia

Koordination: Prof. Dr. Markus Nöthen

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Nöthen	Markus	Prof. Dr. med.	Universitätsklinikum Bonn	4a	Hochdurchsatz-Genotypisierung
Bettecken	Thomas	Dr. rer. nat.	Max Planck Institut für Psychiatrie	4b	Hochdurchsatz-Genotypisierung
Rietschel	Marcella	Prof. Dr. med.	Zentralinstitut für Seel. Gesundheit	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Reinelt	Gerhard	Prof. Dr. med.	Universität Heidelberg	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Schulze	Thomas G.	PD Dr. med.	Unit on the Genetic Basis of Mood and Anxiety Disorders	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Meyer-Lindenberg	Andreas	Prof. Dr. med. Dipl. math.	Zentralinstitut für Seelische Gesundheit	6a	Imaging Genetik
Walter	Henrik	Prof. Dr. med. Dr. phil.	Universitätsklinikum Bonn	6b	Imaging Genetik
Heinz	Andreas	Prof. Dr. med.	Charité–Universitätsmedizin Berlin	6c	Imaging Genetik
Wienker	Thomas F.	Prof. Dr. med.	Universitätsklinikum Bonn	7	Statistische Analysen zu genomweiten Assoziationsstudien
Müller-Myhsok	Bertram	Prof. Dr. med.	Max Planck Institut für Psychiatrie	8	Entwicklung statistischer Methoden für komplexe Gen-Gen Interaktionen in genomweiten Datensätzen
Cichon	Sven	PD Dr. rer. nat.	Universitätsklinikum Bonn	9	Allel-spezifische Expression
Becker	Albert	Prof. Dr. med.	Universitätsklinikum Bonn	9	Allel-spezifische Expression
Eils	Roland	Prof. Dr.	DKFZ Heidelberg	10	Methodenentwicklung für biologische Pathway-Informationen in GWA-Analysen
Brors	Benedikt	Dr. rer. nat.	Universität Heidelberg	10	Methodenentwicklung für biologische Pathway-Informationen in GWA-Analysen

Wanker	Erich E.	Prof. Dr. rer. nat.	Max-Delbrueck-Center für Molekulare Medizin Berlin-Buch	11	Protein-Protein Interaktions-Netzwerk
Zimmer	Andreas	Prof. Dr. rer. nat.	Universitätsklinikum Bonn	12a	Funktionelle Untersuchungen mit Hilfe von transgenen Maus-modellen und Proteomanalysen
Wurst	Wolfgang	Prof. Dr. rer. nat.	Helmholtz Zentrum München	12b	Funktionelle Untersuchungen mit Hilfe von transgenen Maus-modellen und Proteomanalysen
Turck	Chris	Prof. Dr. rer. nat.	Max Planck Institut für Psychiatrie	12	Funktionelle Untersuchungen mit Hilfe von transgenen Mausmodellen und Proteomanalysen
Nöthen	Markus	Prof. Dr. med.	Universitätsklinikum Bonn	14	Projekt-Management und Graduierten-Training
Raff	Ruth	Dr. rer. nat.	Universitätsklinikum Bonn	14	Projekt-Management und Graduierten-Training

IG Genetics of Alcohol Addiction

Koordination: Prof. Dr. Rainer Spanagel

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Spanagel	Rainer	Prof.Dr.	Central Institute of Mental Health	TP1	Coordination Consortium
Eils	Roland	Prof.Dr.	DKFZ	TP2	Gene data mining platform and statistics
Brors	Benedikt	Dr.	German Cancer Research Center	TP2	Gene data mining platform and statistics
Wienker	Thomas	Prof.Dr.	University of Bonn	TP2	Gene data mining platform and statistics
Matthäus	Franziska	Dr.	University of Heidelberg,	TP3	Mathematical Modelling and Analysis
Jäger	Willi	Prof. Dr. Dr. h.c. mult	University of Heidelberg,	TP3	Mathematical Modelling and Analysis
Schütz	Günter	Prof. Dr. med.	German Cancer Research Center (DKFZ)	SP4	Functional analysis I and conditional mouse models
Wurst	Wolfgang	Prof.Dr.	GSF - National Research Center for Environment and Health,	TP5	Functional analysis II and RNAi in vivo application
Deussing	Jan	Dr.	Max Planck Institute of Psychiatry	TP5	Functional analysis II and RNAi in vivo application
Zimmer	Andreas	Prof.Dr.	University of Bonn	TP2b,TP6, TP13a	Functional analysis III
Bartsch	Dusan	Prof.Dr.	Central Institute of Mental Health	TP7	Transgenic rat models
Spanagel	Rainer	Prof.Dr.	Central Institute of Mental Health	TP8	
Gebicke-Haerter	Peter	Prof.Dr.	Central Institute of Mental Health	TP9a	Glutamatergic and epigenetic profiling with microarrays
Hoheisel	Jörg	Dr.	DKFZ	TP9b	Glutamatergic and epigenetic profiling with microarrays

Sprengel	Rolf	Dr.	MPI Med. Forschung Heidelberg	TP10	Transcriptional and posttranscriptional modifications
Rietschel	Marcella	Prof.Dr.	Central Institute of Mental Health	TP11a	GWA studies in alcohol dependent patients and replication studies
Nöthen	Markus	Prof.Dr.	University of Bonn	TP11b	GWA studies in alcohol dependent patients and replication studies
Dahmen	Norbert	PD Dr.	Universität Mainz	TP12a	GWA studies in population-based samples for high versus low alcohol consumption and replication studies
Wichmann	H.Erich	Prof.Dr.	GSF Institute of Epidemiology	TP12b	GWA studies in population-based samples for high versus low alcohol consumption and replication studies
Heinz	Andreas	Prof.Dr.	University Medical Center Berlin, Campus Charité	SP13b	Endophenotyping with fMRI: Genetic modulation and treatment response
Walter	Henrik	Prof.Dr.	University Clinic Bonn,	SP13	Endophenotyping with fMRI: Genetic modulation and treatment response
Kiefer	Falk	Prof.Dr.	Central Institute of Mental Health	SP13c	Endophenotyping with fMRI: Genetic modulation and treatment response
Mann	Karl	Prof.Dr. Dr.	Central Institute of Mental Health	SP14	Endophenotyping with spectroscopy: Genetic modulation and treatment response
Ende	Gabriele	Dr.	Central Institute of Mental Health	SP14	Endophenotyping with spectroscopy: Genetic modulation and treatment response
Gallinat	Jürgen	PD Dr.	Psychiatry, Charité, CCM	SP14	Endophenotyping with spectroscopy: Genetic modulation and treatment response
Sartorius	Alexander	Dr.	Central Institute of Mental Health	SP15	Glutamate spectroscopy at 9.4T combined with microdialysis in rodents

IG German Mental Retardation Network (Netzwerk Mentale Retardierung)

Koordination: Prof. Dr. André Reis

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Rauch	Anita	Prof. Dr. med.	Friedrich-Alexander-Universität Erlangen-Nürnberg	1	MR Zentrum Erlangen
Ropers	Hans-Hilger	Prof. Dr. med.	Max Planck Institut für Molekulare Genetik	2	MR Zentrum Berlin
Riess	Olaf	Prof. Dr. med.	Eberhard-Karls-Universität Tübingen	3	MR Zentrum Tübingen
Strom	Tim M	PD Dr. med.	Helmholtz Zentrum München	4	MR Zentrum München

Engels	Hartmut	Dr. rer. nat.	Rheinische Friedrich-Wilhelms-Universität Bonn	5	MR Zentrum Bonn
Wieacker	Peter	Prof. Dr. med.	Medizinische Fakultät der Westfälischen Wilhelms-Universität Münster	6	MR Zentrum Münster
Schröck	Evelin	Prof. Dr. med.	Medizinische Fakultät Carl Gustav Carus der Technischen Universität Dresden	7	MR Zentrum Dresden
Wieczorek	Dagmar	PD Dr. med.	Universität Duisburg Essen	8	MR Zentrum Essen
Rappold	Gudrun	Prof. Dr. rer. nat.	Ruprechts-Karls Universität Heidelberg	9	MR Zentrum Heidelberg
Schenck	Annette	Dr. rer. nat.	Radboud Universität Nijmegen	10	Modellierung mentaler Retardierung in Fliegen
Reis	André	Prof. Dr. med.	Friedrich-Alexander-Universität Erlangen-Nürnberg	11	Projektkoordination

IG Epilepsy and Migraine Integrated Network (EMINet)

Koordination: Prof. Dr. Christian Kubisch

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Kubisch	Christian	Prof. Dr.	University of Cologne	1	Genome-wide association analysis and gene identification in migraine with aura (TP1)
Dichgans	Martin	Prof. Dr.	LMU Munich	2	Whole-genome association study in migraine without aura and functional characterization of disease associated alleles (TP2)
Sander	Thomas	Dr.	University of Cologne	3	Genome-wide association mapping of gene configurations conferring risk to idiopathic generalized epilepsies (TP3)
Nürnberg	Peter	Prof. Dr.	University of Cologne	4	High-throughput sequencing of functional and positional candidate genes for common forms of migraine and epilepsy (TP4)
Schoch-McGovern	Susanne	Dr.	University of Bonn	5	Genetic basis of Levetiracetam pharmacoresistance and side effects in human epilepsy (TP5)
Lerche	Holger	Prof. Dr.	University of Ulm	6	Functional analysis of human ion channel mutations in cellular and animal models (TP6)
Becker	Albert	PD Dr.	University of Bonn	7	Aberrant transcriptional networks in human epileptic tissue

Beck	Heinz	Prof. Dr.	University of Bonn	8	Mechanisms underlying the development of cellular hyperexcitability in mouse models of human epilepsy
Isbrandt	Dirk	Dr.	University of Hamburg	9	Subthreshold ion channels in epileptogenesis and neuronal synchronization

IG Gene Identification and Functional analyses in Alzheimer's disease

Koordination: PD Dr. Matthias Riemenschneider

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Riemenschneider	Matthias	PD Dr	Universitätsklinikum des Saarlandes	1	Identification of genetic factors in Alzheimer's disease
Krobitsch	Sylvia	Dr	Max Plank Institut für molekulare Genetik	2	Identification and functional characterization of novel early-onset Alzheimer's genes
Haass	Christian	Prof. Dr.	LMU München	3a	The physiological function of BACE1-is BACE1 a safe therapeutic target?
Garratt	Alistair	Dr	Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch	3b	The physiological function of BACE1-is BACE1 a safe therapeutic target?
Müller	Ulrike	Prof. Dr.	University of Heidelberg	4	In vivo analysis of APP functional domains-can we safely abrogate APP/APLP processing?
Hartmann	Tobias	Prof. Dr.	Universität des Saarlandes	5	Functional involvement of Alzheimer's disease candidate risk genes in lipid homeostasis, Ab metabolism and Ab response
Endres	Kristina	Dr.	Johannes Gutenberg Univers. Mainz	6	Regulation of ADAM10 gene expression and neuroprotection
Jucker	Mathias	Prof. Dr.	Hertie-Institut für klinische Hirnforschung	7	Pathomechanism of Cerebral Amyloid Angiopathy
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch	8	Identification and characterization of modulators of Alzheimer's disease pathogenesis
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	9	Animal models for candidate genes of Alzheimer's disease
Riemen-schneider	Matthias	PD Dr	Universitätsklinikum des Saarlandes	10	Scientific administration office of the AD-IG

IG Functional Genomics of Parkinson's disease

Koordination: Prof. Dr. Thomas Gasser

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Gasser	Thomas	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP1/TP2	Scientific Coordinating Office
Klein	Christine	Prof. Dr.	Universität Lübeck	TP3	Mutations in recessive Parkinson's

					disease genes
Höglinger	Günther	PD. Dr.	Philipps-Universität Marburg	TP4	Genome-wide siRNA screen in an α-synuclein-based in vitro model of Parkinson's disease
Schulz	Jörg B.	Prof. Dr.	Universität Aachen	TP5, TP6a	Modifier screen in flies overexpressing LRRK2
Zweckstetter	Markus	Prof. Dr.	Universität Göttingen	TP6b	Molecular mechanisms of pathogenic misfolding of α-synuclein
Auburger	Georg	Prof. Dr.	J.W. Goethe University	TP7	Biomarkers of the common Parkinson pathway:α-Synuclein induction and synaptic pathology in recessive PD
Riess	Olaf	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP8	Calpain cleavage of α-synuclein in the pathogenesis of Parkinson's disease by cell culture and animal models
Kahle	Philipp	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP9	Regulation of Apoptosis Signal Regulating Kinase Pathways by DJ-1 and Parkin
Krüger	Rejko	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP10	Mitochondrial stress response in neurodegeneration and aging: OMI and DJ-1 mediated signalling pathways
Haass	Christian	Prof. Dr.	Ludwig-Maximilians-Universität München	TP11	The physiological and pathological function of PINK 1, parkin and LRRK2 in zebrafish and other models
Winklhofer	Konstanze	PD. Dr.	Ludwig-Maximilians-Universität München	TP11	The physiological and pathological function of PINK 1, parkin and LRRK2 in zebrafish and other models
Ueffing	Marius	PD. Dr.	TU München	TP12	Functional characterization of LRRK2 in mammalian cells and tissues
Roeper	Jochen	Prof. Dr.	J.W. Goethe University	TP13a	Dopaminergic dysfunction and molecular pathways to selective neurodegeneration: from mouse models to Parkinson disease.
Liss	Birgit	Prof. Dr.	Universität Ulm	TP13b	Dopaminergic dysfunction and molecular pathways to selective neurodegeneration: from mouse models to Parkinson disease.
Schütz	Günther	Prof. Dr.	German Cancer Research Center	TP14	Characterization of genetic mouse models for Parkinson´s disease
Wurst	Wolfgang	Prof. Dr.	Institut für Entwicklungsgenetik, Helmholtz Zentrum München	TP14	Characterization of genetic mouse models for Parkinson´s disease
Marcus	Katrin	Prof. Dr.	Ruhr University Bochum	TP15	Core facility: High-performance proteome analysis for biomarker discovery and elucidation of pathomechanisms
Zell	Andreas	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP16	Core facility: Bioinformatics: data integration towards a systems level model of Parkinson's disease Generation of a systems biology

					model
Meitinger	Thomas	Prof. Dr.	Helmholtz Zentrum München	Core facility	Core facility: High throughput genotyping

IG NeuroNet - Verbundprojekt Neurodegeneration
Koordination:
Prof. Dr. Erich Wanker

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	1	Protein-Protein Interaktionsnetzwerke in neurodegenerativen Erkrankungen
Selbach	Matthias	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	2	Protein Interaktionsscreening durch quantitative Massen-spektroskopie
Stelzl	Ulrich	Dr.	Max-Planck-Institut für Molekulare Genetik	3	Modulation von Protein-Protein Wechselwirkungen durch Phosphorylierung
Priller	Josef	Prof. Dr.	Charité - Universitätsmedizin Berlin	4	Klassifikation von Phänotyp-Genotyp-Beziehungen bei - neurodegenerativen Erkrankungen
Lange	Bodo	PD Dr.	Max-Planck-Institut für Molekulare Genetik	5	Modulation von Proteinkomplex-komposition und Funktion durch Stress und eurodegenerative Krankheitssignale
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	6a	Erstellung von Genexpressions-signaturen von neuro-degenerativen Krankheits-prozessen
Nietfeld	Wilfried	Dr.	Max-Planck-Institut für Molekulare Genetik	6b	Erstellung von Genexpressions-signaturen von neuro-degenerativen Krankheits-prozessen
Bork	Peer	Prof. Dr.	European Molecular Biology Laboratory	6c	Erstellung von Genexpressions-signaturen von neuro-degenerativen Krankheits-prozessen
Boutros	Michael	Prof. Dr.	Deutsches Krebsforschungszentrum	7	Systematische Analyse von Phänotypen mittels RNAi und kleinen Molekülen
Bork	Peer	Prof. Dr.	European Molecular Biology Laboratory	8a	Datenintegration und Erstellung von Phänotyp-Protein-Wirkstoff Netzwerken
Andrade	Miguel	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	8b	Datenintegration und Erstellung von Phänotyp-Protein-Wirkstoff Netzwerken

Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	9	Management der IG „NeuroNet“
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin		Wissenschaftliche Plattform „Interaktom“ für systematische Protein-Interaktionsstudien

IG From Disease genes to Protein Pathways (DiGTOP)

Koordination: Prof. Dr. Wolfgang Wurst

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Stewart	Francis	Prof. Dr.	Technische Universität Dresden	TP1	Genidentifikation und DNA Konstruktproduktion
von Melchner	Harald	Prof. Dr.	Universität Frankfurt	TP2	In situ Markierung von Krankheitsproteinen in embryonalen Stammzellen mit Genfallen-induzierten Mehrzweckallelen
Wurst	Wolfgang	Prof. Dr.	HelmholtzZentrum München	TP3	Produktion proteinmarkierter pluripotenter und differenzierter ES Zellen
Hyman	Tony	Prof. Dr.	MPI für Zellbiologie und Genetik Dresden	TP4	Produktion und Imaging von HeLa und ES Zelllinien
Brüstle	Oliver	Prof. Dr.	Universität Bonn	TP5	Etablierung und Analyse transgener hES Zelllinien und neuralen Stammzelllinien
Mann	Matthias	Prof. Dr.	MPI für Biochemie, Martinsried	TP6	Proteininteraktionsstudien mittels massenspektrometriebasierter Proteomik in in vitro und in vivo Systemen
Hansen	Jens	Dr.	HelmholtzZentrum München	TP7a	DiGtoP bioinformatics – resource development
Gibson	Toby	Prof. Dr.	EMBL Heidelberg	TP7	DiGtoP bioinformatics – resource application in comparative network analysis
Kühn	Ralf	Dr.	HelmholtzZentrum München	TP8	Mausmodelle für die in vivo Validierung von Proteininteraktionen
Buchholz	Frank	Dr.	MPI für Zellbiologie und Genetik Dresden	TP9	Validierung und Zergliederung der Signalwege von Krankheitsrelevanten Genen mit endoribonucelase präparierter siRNA
Wurst	Wolfgang	Prof. Dr.	HelmholtzZentrum München	TP10	Management & Training

IG German Mouse Clinic (GMC)

Koordination: Prof. Dr. Martin Hrabě de Angelis

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Hrabé de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München		

Gailus-Durner	Valérie	Dr.	Helmholtz Zentrum München		
Hrabé de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	1	Core Facility
Gailus-Durner	Valérie	Dr.	Helmholtz Zentrum München	1	Core Facility
Fuchs	Helmut	Dr.	Helmholtz Zentrum München	1	Core Facility
Gailus-Durner	Valérie	Dr.	Helmholtz Zentrum München	1	Core Facility
Wolf	Eckhard	Prof.	Genzentrum der LMU München	2	Clinical Chemical Screen
Rathkolb	Birgit	Dr.	Genzentrum der LMU München	2	Clinical Chemical Screen
Wurst	Wolfgang	Prof.	Helmholtz Zentrum München	3	Behavioral Screen
Hölter-Koch	Sabine	Dr.	Helmholtz Zentrum München	3	Behavioral Screen
Hölter-Koch	Sabine	Dr.	Helmholtz Zentrum München	3	Behavioral Screen
Klopstock	Thomas	PD Dr. med.	LMU München	4	Neurological Screen
Becker	Lore	Dr.	LMU München	4	Neurological Screen
Graw	Jochen	Prof.	Helmholtz Zentrum München	5	Eye Screen
Puk	Oliver	Dr.	Helmholtz Zentrum München	5	Eye Screen
Hrabé de Angelis	Martin	Prof.	Helmholtz Zentrum München	6	Dysmorphology Screen
Fuchs	Helmut	Dr.	Helmholtz Zentrum München	6	Dysmorphology Screen
Fuchs	Helmut	Dr.	Helmholtz Zentrum München	6	Dysmorphology Screen
Busch	Dirk	Prof.	TU München	7	Immunology Screen
Adler	Thure	Dr.	TU München	7	Immunology Screen
Ollert	Markus	Prof.	TU München	8	Allergy Screen
Aguilar	Antonio	Dr.	TU München	8	Allergy Screen
Adamski	Jerzy	Prof.	Helmholtz Zentrum München	9	Steroid Screen
Prehn	Cornelia	Dr.	Helmholtz Zentrum München	9	Steroid Screen
Zimmer	Andreas	Prof.	Universitätsklinikum Bonn	10	Nociceptive Screen
Schulz	Holger	Prof.	Helmholtz Zentrum München	11	Lung Function Screen
Stöger	Tobias	Dr.	Helmholtz Zentrum München	11	Lung Function Screen
Götz	Alexander	Dr.	Helmholtz Zentrum München	11	Lung Function Screen
Beckers	Johannes	PD Dr.	Helmholtz Zentrum München	12	Molecular Phenotyping Screen
Horsch	Marion	Dr.	Helmholtz Zentrum München	12	Molecular Phenotyping Screen
Klingenspor	Martin	Prof.	TU München	13	Energy Metabolism Screen
Daniel	Hannelore	Prof.	TU München	13	Energy Metabolism Screen
Rozman	Jan	Dr.	TU München	13	Energy Metabolism Screen

Katus	Hugo	Prof.	Universität Heidelberg	14	Cardiovascular Screen
Ivandic	Boris	Dr.	Universität Heidelberg	14	Cardiovascular Screen
Schrewe	Anja	Dr.	Helmholtz Zentrum München	14	Cardiovascular Screen
Höfler	Heinz	Prof.	Helmholtz Zentrum München	15	Pathology Screen
Esposito	Irene	PD Dr.	Helmholtz Zentrum München	15	Pathology Screen
Esposito	Irene	PD Dr.	Helmholtz Zentrum München	15	Pathology Screen
Hrabé de Angelis	Martin	Prof.	Helmholtz Zentrum München	16	Data Management
Lengger	Christoph	Dr.	Helmholtz Zentrum München	16	Data Management
Lengger	Christoph	Dr.	Helmholtz Zentrum München	16	Data Management
Schughart	Klaus	Prof.	HZI - Helmholtz-Zentrum für Infektionsforschung	17	Host Pathogen Interaction Screen
Hrabé de Angelis	Martin	Prof.	Helmholtz Zentrum München	18	EMMA
Hagn	Michael	Dr.	Helmholtz Zentrum München	18	EMMA
Hagn	Michael	Dr.	Helmholtz Zentrum München	18	EMMA
IG MHC Haplotype Sequencing: An Integrated Approach to Common Disease					
Koordination: Dr. Margret Hoehe					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Hoehe	Margret	Dr.	MPI-MG Berlin	TP1	MHC-Haplotypen-Sequenzierung
IG Cellular Systems Genomics in Health and Disease Koordination: PD Dr. Stefan Wiemann					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	TP1	**Coordinating office**
Bechtel	Stefanie	Dr.	DKFZ Heidelberg	TP2	Functional Genomic Resources for NGFNplus
Tschulena	Ulrich	Dr.	DKFZ Heidelberg	TP3	Cellular Screening Systems
Arlt	Dorit	Dr.	DKFZ Heidelberg	TP4	Signalling Network analysis
Gavin	Anne-Claude	Dr.	EMBL Heidelberg	TP5	TAP - Protein interaction mapping
Pepperkok	Rainer	Dr.	EMBL Heidelberg	TP6	Protein and Network dynamics
Korf	Ulrike	Dr.	DKFZ Heidelberg	TP7	Quantitative Proteinarrays
Lange	Bodo	PD Dr.	Max-Planck Institut für Molekulare Genetik	TP8	Primary Cancer Cell Models
Schneeweiss	Andreas	Prof. Dr.	Uniklinik Heidelberg	TP9	Clinical validation

Beissbarth	Tim	Dr.	DKFZ Heidelberg	TP10	Pathway reconstruction & modelling
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	TP10	Pathway reconstruction & modelling
Rosenfelder	Heiko		DKFZ Heidelberg	TP11	Integrated bioinformatics
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	TP12	QM & Standards

IA Entwicklung prophylaktisch wirksamer Anti-Malaria Verbindungen

Koordination:
Dr. Birte Sönnichsen

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Sönnichsen	Birte	Dr.	Cenix BioScience GmbH	1	Anti Malaria Zielgene und Wirkstoffkandidaten
Matuschewski	Kai	PD Dr.	Max-Planck-Institut für nfektionsbiologie	2	Zielgene im Parasiten
Frischknecht	Friedrich	Dr.	Universität Heidelberg	3	Imaging von interaktionen des Parasiten mit Leberzellen

IA Breast Cancer Kit

Koordination: Prof. Dr. Jan Georg Hengstler

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Schmidt	Marcus	Dr. med.	Universität Mainz	1	
Gehrmann	Mathias	Dr.	Siemens Medical Solutions Diagnostic GmbH	2	
Hengstler	Jan Georg	Prof. Dr. med.	Institut für Arbeitsforschung an der Technischen Universität Dortmund	3	Oncoprofile-Kit

IA Heart Failure Therapy

Koordination:
Prof. Dr. Markus Hecker

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Grupe	Helga	Dr.	AVONTEC GmbH	1	
Berghöfer	Beate	Dr.	AVONTEC GmbH	1	
Hecker	Markus	Prof. Dr.	Universität Heidelberg	2	
Wagner	Andreas H.	PD Dr.	Universität Heidelberg	2	
Müller	Oliver J.	PD Dr. med.	Universität Heidelberg	3	
Bekeredjian	Raffi	PD Dr. med.	Universität Heidelberg	3	

IA Metabolomics in Heart Failure as a Novel Diagnostic Tool					
Koordination: Prof. Dr. Hugo A. Katus					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	1a	Novel Biomarkers for Heart Failure - Metabolic Signatures (Erster Projektleiter und Ansprechpartner auf der Arbeitsebene)
Fuhrmann	Jens	Dr.	metanomics GmbH	1b	Novel Biomarkers for Heart Failure - Metabolic Signatures (Co-Pi und Ansprechpartner auf der Arbeitsebene)
Frey	Norbert	PD Dr.	Universitätsklinikum Schleswig-Holstein, Campus Kiel	2	Metabolic Profiling in Mouse Models of Heart Failure (Erster Projektleiter und Ansprechpartner auf der Arbeitsebene)
Müller	Oliver	Dr.	Universitätsklinikum Heidelberg	2	Metabolic Profiling in Mouse Models of Heart Failure (Co-Pi und Ansprechpartner auf der Arbeitsebene)
Weis	Tanja	Dr.	Universitätsklinikum Heidelberg		Coordination
IA New Tools for the Prevention of Cardiovascular Diseases and Disorders in Chronic Kidney Disease					
Koordination: Prof. Dr. Joachim Jankowski					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Jankowski	Vera	Dr.	Charité – Universitätsmedizin Berlin	1	Bioanalytik der chronischen Niereninsuffizienz
Lehmann	Kerstin	Dr.	Charité – Universitätsmedizin Berlin	2	Effekte auf aktivierte Endothelzellen
Buschmann	Ivo	PD Dr.	Charité – Universitätsmedizin Berlin	2	Effekte auf aktivierte Endothelzellen
Herget-Rosenthal	Stefan	PD Dr.	Universitätsklinikum Essen / Universität Duisburg Essen	3	Patienten und Proben
Lehrach	Hans	Prof. Dr.	MPIMG	4	Bioinformatik
Herwig	Ralf	Dr.	MPIMG	4	Bioinformatik
Lemke	Horst-Dieter	Dr.	EXcorLab GmbH	5	Aktivierung von Neutrophilen durch urämische Proteine
Krahn	Thomas	Dr.	Bayer Schering Pharma	6	CVD Drug Discovery Biomarker & Targets

IA Proteinanalysen in FFPE Brustkrebsgeweben - Brustkrebsmarker

Koordination: Prof. Dr. Karl-Friedrich Becker

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Becker	Karl-Friedrich	Prof. Dr.	Technische Universität München	TP 1	Proteinlysat Mikroarrayanalyse für uPA und PAI-1 von Formalin-fixierten Brustkrebsgeweben
				TP2	HER2-Rezeptor Expression und Signalwege in Brustkrebsgeweben
Porschewski	Peter	Dr.	Qiagen GmbH	TP3	Proteomsignaturen in FFPE-Geweben

IA Subgenome Fraktionation for High Throughput Sequencing

Koordination: Dr. Bernhard Korn

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Beier	Markus	Dr.	febit AG	TP1	Development of microarrays for subgenome preparation
Korn	Bernhard	Dr.	DKFZ-Heidelberg	TP2	Cancer Genome Comparisons
Scharfenberger-Schmeer	Maren	Dr.	DKFZ	TP2	Cancer Genome Comparisons
Pfeufer	Arne	PD Dr.	TU München	TP3a	Cardiomyopathy Re-sequencing
Katus	Hugo	Prof.Dr.	Universitätsklinik Heidelberg	TP3b	Cardiomyopathy Re-sequencing
Meder	Benjamin	Dr.	Universitätsklinik Heidelberg	TP3b	Cardiomyopathy Re-sequencing
Strom	Tim	Dr.	Helmholtzzentrum München	TP4	Coverage and variation detection

IA Whole Genome and Transcriptome Amplification in Large Biobanks

Koordination: Prof. Dr. Dr. H.-Erich Wichmann,
Dr. Christian Korfhage

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Teilprojekt Titel**
Korfhage	Christian	Dr.	Qiagen	1	Development and standardization of new WGA and WTA methods
Wichmann	H.-Erich	Prof.Dr. Dr.	HMGU	2	Provision of biosamples of different quality to test whole genome and transcriptome amplification techniques.
Klopp	Norman	Dr.	HMGU	2	Provision of biosamples of different quality to test whole genome and transcriptome amplification techniques.
Wichmann	H.-Erich	Prof.Dr. Dr.	HMGU	3	Transfer of the results to international organisations in the field of biobanking

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