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Welcome Remarks

Dear conference participant,

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

5th Annual Meeting of NGFN-Plus and NGFN-Transfer
in the Program of Medical Genome Research
11th – 13th December 2012 at DKFZ, Heidelberg.

At this year's event we are pleased to announce a highly international program featuring exceptional speakers from large-scale genome projects which are affiliated with the NGFN. The conference comprises outstanding scientists in the field of medical genome research. It provides the opportunity to present scientific results and the exchange of latest developments, as well as discussion and interaction with a number of competent international researchers in a highly dynamic, pleasant atmosphere.

The conference starts on December 11 with two **Satellite Symposia** that focus on *Small RNAs* and *Next-Generation Sequencing*.

The first **two symposia** of the main program on December 11 and 12 are dedicated to internationally renowned speakers presenting latest results of **International Projects in Genome Research** such as the *International Cancer Genome Consortium* and the *International Human Epigenome Consortium*.
The following **four symposia** on December 12 and 13 entitled **Genomics of Common Disease I and II, Functional Genomics,** and **Personalized Medicine** cover the broad scientific spectrum of the NGFN. In concert with invited keynote speakers, **scientists of the NGFN** will present and discuss their latest results that have been selected from submitted abstracts.

The scientific **poster sessions** accompanying symposia 3 to 6 provide an important platform for discussion and networking. In memory of Prof. Dr. Annemarie Poustka and in order to motivate young scientists to present and discuss their newest results, three posters will receive the "**Annemarie Poustka Poster Award** for Medical Genome Research 2012" sponsored by Roche Diagnostics Deutschland GmbH. Annemarie Poustka made outstanding achievements in the field of Genome Research and was a visionary scientist for the NGFN.

Company satellite sessions round out the program and an industrial exposition offers comprehensive information on latest technology developments with relevance for the genome research community.

The **Evening Lecture** given by Prof. Randolph Nesse on Evolutionary Medicine will be a terrific highlight.

Don't miss the **Get-Together** on Wednesday night with good wine, tasty fingerfood and great live music, enjoy the genome research community in a relaxed ambience!

We cordially welcome members of all funding phases of the National Genome Research Network as well as all scientists interested in the program of our conference and explicitly invite you to actively participate in the scientific discussion and idea exchange. To all participants, this is a great opportunity for meeting each other within the BMBF Program of Medical Genome Research, to reinforce existing co-operations, to find new collaborators for upcoming projects and to discuss the future focus of genome research in Germany.

Bonn and Munich, December 2012

Prof. Dr. Markus Nöthen

Prof. Dr. Wolfgang Wurst

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

Conference Management

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Scientific Program Committee

Prof. Dr. Jürgen Brosius
Universität Münster

Prof. Dr. Martin Hrabě de Angelis
Helmholtz Zentrum München

Prof. Dr. Hugo A. Katus
Universitätsklinik Heidelberg

Prof. Dr. Peter Lichter
DKFZ Heidelberg

Prof. Dr. Markus Nöthen
Friedrich-Wilhelms Universität Bonn

Prof. Dr. Matthias Riemenschneider
Universität des Saarlandes

Prof. Dr. Stefan Schreiber
Universitätsklinikum Schleswig-Holstein,
Campus Kiel

Prof. Dr. Roman Thomas
Universität zu Köln

Prof. Dr. Wolfgang Wurst
Helmholtz Zentrum München

Program-at-a-glance

Tuesday, December 11th

12.00 – 02.00 pm	**Satellite Symposium**: Small RNAs (Org. Jürgen Haas and Jürgen Brosius)
02.00 – 02.30 pm	**Coffee Break**
02.30 – 04.00 pm	**Satellite Symposium**: Next-Generation Sequencing (Org. Stefan Wiemann and Bernd Timmermann)
04.00 – 04.30 pm	**Coffee Break**
04.30 – 05.00 pm	***Welcome***
05.00 – 07.00 pm	**Symposium I - International Projects in the Program of Medical Genome Research** Lynda Chin - Peter Lichter - Matthew Meyerson - Hans Lehrach
07.00 pm	**Supper**

Wednesday, December 12th

09.00 – 10.30 am	**Symposium II – International Projects in Genome Research** John Blangero - Joris A. Veltmann - Gerard D. Schellenberg
10.30 – 11.00 am	**Coffee Break**
11.00 – 12.00 am	**Symposium II – International Projects in Genome Research** Peter Jones - Jörn Walter
12.00 – 02.00 pm	***Lunch Break and Poster Session I*** **Company Satellite Sessions** Affymetrix - Caliper a Perkin Elmer Company - Illumina - Bio-Rad Laboratories
02.00 – 03.30 pm	**Symposium II – International Projects in Genome Research** Wolfgang Wurst - Martin Hrabě de Angelis - Matthias Mann
03.30 – 4.00 pm	**Coffee Break**
04.00 – 05.00 pm	**Symposium III – Genomics of Common Disease I** André Reis - Vanessa Nieratschker - Andre Franke - Robert Häsler
05.00 – 05.30 pm	**Coffee Break**
05.30 – 06.00 pm	**Symposium III – Genomics of Common Disease I** Anke Hinney - Ralf Herwig
06.00 – 07.00 pm	**Evening Lecture:** Randolph Nesse
07.00 – 10.00 pm	***Get-Together (Wine, Cheese, Live Music)***

Thursday, December 13th

09.00 - 10.15 am	**Symposium IV – Genomics of Common Disease II** Hugo A. Katus (Keynote) - Christina Loley - Martin Peifer - Christel Herold-Mende
10.15 – 10.45 am	**Coffee Break**
10.45 – 12.00 pm	**Symposium V – Functional Genomics** Jan Korbel - Gerrit Erdmann - Sven Lindner - Sören Westphal - Zouhair Aherrahrou
12.00 - 02.00 pm	***Lunch Break and Poster Session II*** **Company Satellite Sessions:** Life Technologies - Roche Diagnostics - SEQUENOM - Nanostring Technologies
02.00 - 02.15 pm	**Ceremony: "Annemarie Poustka Poster Award 2012" sponsored by Roche Diagnostics Deutschland GmbH**
02.15 - 03.45 pm	**Symposium VI: Personalized Medicine** Rudi Balling (Keynote) - Nicole Teichmann - Margret Hoehe - Jeanette Erdmann - Brajesh Kaistha
03.45 - 04.00 pm	***Concluding Remarks***
04.00 - 04.30 pm	**Closing Coffee**

Program

Tuesday, December 11, 2012

Satellite Symposia - Krehl Klinik Auditorium, Im Neuenheimer Feld 410

12.00 – 02.00 pm	**Satellite Symposium**: Small RNAs (Org. Jürgen Haas and Jürgen Brosius)
02.00 – 02.30 pm	**Coffee Break**
02.30 – 04.00 pm	**Satellite Symposium**: Next-Generation Sequencing (Org. Stefan Wiemann and Bernd Timmermann)
04.00 – 04.30 pm	**Coffee Break** (DKFZ Communication Center)

Welcome - DKFZ Communication Center, Main Auditorium

04.30 – 05.00 pm	**Wolfgang Wurst**, Helmholtz Zentrum Munich, Germany, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research
	N.N., Federal Ministry of Education and Research, Germany
	Christof von Kalle, National Center for Tumor Diseases (NCT)/ German Cancer Research Center (DKFZ), Heidelberg, Germany

Symposium I: International Projects in the Program of Medical Genome Research

05.00 – 05.30 pm	*International Cancer Genome Consortium (ICGC)* **Lynda Chin**, The University of Texas, MD Anderson Cancer Center, Houston, USA *Translation of the Cancer Genome*
05.30 – 06.00 pm	*International Cancer Genome Consortium (ICGC)* **Peter Lichter**, German Cancer Research Center, Heidelberg, Germany *Novel findings in pediatric brain tumors revealed by high resolution genome, methylome and transcriptome analyses*
06.00 – 06.30 pm	*International Cancer Genome Consortium (ICGC)* **Matthew Meyerson**, Dana Farber Cancer Institute, Boston, USA *Genome Alterations in Human Lung Cancers*
06.30 – 07.00 pm	*1000 Genomes Project* **Hans Lehrach,** MPI for Molecular Genetics, Berlin, Germany *1000 Genomes Project – An integrated map of genetic variation*
07.00 pm	**Supper**

Wednesday, December 12, 2012

Symposium II: International Projects in Genome Research

09.00 – 9.30 am **John Blangero,** Texas Biomedical Research Institute, San Antonio, USA
Identification of Complex Disease Genes Using Whole Genome Sequencing in Large Pedigrees

09.30 – 10.00 am **Joris A. Veltman**, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
De novo diagnostics in patients with intellectual disability

10.00 – 10.30 am *Alzheimer's Disease Genetics Consortium (ADGC)*
Gerard D. Schellenberg, University of Pennsylvania School of Medicine, Philadelphia, USA
Neurodegenerative disease genetics; GWAS, exomes and beyond

10.30 – 11.00 am ***Coffee Break***

11.00 – 11.30 am *International Human Epigenome Consortium (IHEC)*
Peter Jones, USC Norris Comprehensive Cancer Center, Los Angeles, USA
The Cancer Epigenome

11.30 – 12.00 pm *International Human Epigenome Consortium (IHEC)*
Jörn Walter, Saarland University, Germany
Control of DNA-methylation in development

12.00 – 02.00 pm ***Lunch Break and Poster Session I***

Company Satellite Sessions

12.10 – 12.40 pm **Maik Pruess, Affymetrix UK Ltd**
From basic research to clinical samples. When are microarrays the right tool for the job?

12.45 – 01.15 pm **Hannes Arnold, Caliper a PerkinElmer Company**
Automation of NGS Sample Preparation: From Benchtop NGS to Genome Centers

01.20 – 01.50 pm **Luc Smink, Illumina UK Ltd**
Illumina's Genomics Research Portfolio: From Whole Genomes to Targeted approaches

01.55 – 02.25 pm **Pia Scheu, Bio-Rad Laboratories GmbH**
Droplet Digital PCR: Molecular Biology in High Resolution

Symposium II:	**International Projects in Genome Research**
02.00 – 02.30 pm	*International Knockout Mouse Consortium / EUCOMM* **Wolfgang Wurst**, Helmholtz Zentrum Munich, Germany *International Mouse Knock-out Consortium: Resource to functionally validate all genes*
02.30 – 03.00 pm	*International Mouse Phenotyping Consortium / EUMODIC* **Martin Hrabě de Angelis**, Helmholtz Zentrum Munich, Germany *Creating a comprehensive encyclopedia of mammalian gene function*
03.00 – 03.30 pm	**Matthias Mann,** Max Planck Institute for Biochemistry, Munich, Germany *High resolution, quantitative mass spectrometry combines proteomics and genomics*
03.30 – 04.00 pm	***Coffee Break***

Symposium III:	**Genomics of Common Disease I**
04.00 – 04.15 pm	**André Reis**, University Erlangen-Nuremberg, Germany *Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: An exome sequencing study*
04.15 – 04.30 pm	**Vanessa Nieratschker**, Central Institute for Mental Health, Mannheim, Germany *Pre-, peri- and postnatal stress in human and non-human off-spring: A convergent approach to study genetic and epigenetic impact on depression*
04.30 – 04.45 pm	**Andre Franke**, University Hospital Schleswig-Holstein, Kiel, Germany *Host-microbe interactions shape genetic risk for inflammatory bowel disease*
04.45 – 05.00 pm	**Robert Häsler,** Christian Albrechts University of Kiel, Germany *Mapping the functional epigenome of Ulcerative Colitis in monozygotic twins*
05.00 – 05.30 pm	***Coffee Break***
05.30 – 05.45 pm	**Anke Hinney**, University of Duisburg-Essen, Germany *Childhood ADHD and obesity: Evidence for a common genetic link*
05.45 – 06.00 pm	**Ralf Herwig**, Max Planck Institute for molecular Genetics, Berlin, Germany *An integrated catalogue of genome, methylome and gene expression variations in colon cancer*

Evening Lecture:	
06.00 – 07.00 pm	**Randolph Nesse**, The University of Michigan, Ann Arbor, USA *Evolutionary Medicine and Molecular Medicine: Synergistic Siblings*
07.00 – 10.00 pm	***Get-Together (Wine, Cheese, Live Music)***

Thursday, December 13, 2012

Symposium IV: Genomics of Common Disease II

09.00 – 09.30 am **Keynote: Hugo A. Katus,** Heidelberg University Hospital, Germany
Innovations in translational research – Successes of NGFN

09.30 – 09.45 am **Christina Loley**, University of Lübeck, Germany
Association of X-chromosomal variants with coronary heart disease: Results from a meta-analysis

09.45 – 10.00 am **Martin Peifer**, University of Cologne, Germany
Toward a novel genomics-based taxonomy of lung cancer: The Clinical Lung Cancer Genome Project

10.00 – 10.15 am **Christel Herold-Mende**, University of Heidelberg, Germany
Aberrant self-renewal and quiescence contribute to Glioblastoma aggressiveness

10.15 – 10.45 am ***Coffee Break***

Symposium V: Functional Genomics

10.45 – 11.00 am **Jan Korbel**, The European Molecular Biology Laboratory, Heidelberg, Germany
Genome sequencing of childhood medulloblastoma brain tumors links chromothripsis with TP53 mutations

11.00 – 11.15 am **Gerrit Erdmann**, German Cancer Research Center, Heidelberg, Germany
Secretion of Wnts is required for Wnt/ß-catenin pathway activity in colorectal cancer (CRC) despite APC or ß-catenin mutations

11.15 – 11.30 am **Sven Lindner**, University Hospital Essen, Germany
*LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression***.**

11.30 – 11.45 am **Sören Westphal**, University Clinic Ulm, Germany
Human BCL2-associated athanogene 3 (BAG3) mutations lead to dilated cardiomyopathy in zebrafish

11.45 – 12.00 pm **Zouhair Aherrahrou**, University of Lübeck, Germany
*Cyp17a1 deficient mice display increased body weight, visceral/subcutaneous fat deposition and altered lipid metabolism***.**

12.00 – 02.00 pm ***Lunch Break and Poster Session II***

Company Satellite Sessions:

12.10 – 12.40 am **Raimo Tanzi, Life Technologies GmbH**
Ion torrent next Generation sequencing technology: the revolution of the revolution

12.45 – 01.15 pm **Janine Altmüller, Roche Diagnostics Deutschland GmbH**
Whole exome sequencing (WES) speeds up gene identification in monogenetic human diseases

01.20 – 01.50 pm **Rebekka Krumbach, Oncotest, Susanne Müller, SEQUENOM GmbH**
Translating Genomic Discovery into Human Health - The MassARRAY® for Somatic Mutation Profiling in Cancer and Quality Control for NGS Projects

01.55 – 02.25 pm **Jim White, Nanostring Technologies**
Gene Expression Analysis Down to the single Cell level by Digital Quantification of Nucleic Acids Utilizing a Color-Coded Barcode Technology

Poster Award Ceremony:

02.00 – 02.15 pm **Ceremony: "Annemarie Poustka Poster Award 2012"** sponsored by Roche Diagnostics GmbH
Christine Kuch, Roche Diagnostics Deutschland GmbH
Hugo A. Katus, Heidelberg University Clinics, Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

Symposium VI: Personalized Medicine

02.15 – 02.45 pm **Keynote: Rudi Balling**, Luxembourg Centre for Systems Biomedicine, Luxembourg
Systems approaches to Parkinson´s disease

02.45 – 03.00 pm **Nicole Teichmann**, University Hospital rechts der Isar, Munich, Germany
A promising therapy strategy for PDAC: MEK1/2 inhibition with the novel chemotherapeutic drug BAY 86-9766 (RDEA119)

03.00 – 03.15 pm **Margret Hoehe**, Max Planck Institute for Molecular Genetics, Berlin, Germany
Haplotype-resolving multiple human genomes: Key to personalized medicine and genome biology

03.15 – 03.30 pm **Jeanette Erdmann**, University of Lübeck, Germany
The risk of myoacardial infarction is increased by digenic mutation in GUCY1A3 and CCT7 - identified by exome sequencing in an extended family

03.30 – 03.45 pm **Brajesh Kaistha**, University of Marburg, Germany
High-throughput cell-based assays identify Placenta-specific 8 (Plac8; Onzin) as a key regulator of proliferation and survival in pancreatic cancer cells

03.45 – 04.00 pm **Concluding Remarks: Markus Nöthen**, Friedrich-Wilhelms University, Bonn, Spokesperson for the Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

04.00 – 04.30 pm **Closing Coffee**

National Genome Research Network

Satellite Symposia

Satellite Symposium
Small RNAs
Organisation: Jürgen Haas and Jürgen Brosius

Krehl Klinik Auditorium, Im Neuenheimer Feld 410

Welcome and Introduction

12.00 – 12.45 pm **Bryan Cullen** (Molecular Genetics and Microbiology, Duke University Medical Center)
Viruses and microRNAs

12.45 – 01.10 pm **Sebastién Pfeffer** (Institut de Biologie Moléculaire et Cellulaire, University of Strasbourg)
Host-virus interactions and microRNAs in mammals

01.10 – 01.35 pm **Gunter Meister** (Biology and preclinical medicine, University of Regensburg)
Analysis of Argonaute interactions in mammalian cells

01.35 – 02.00 pm **Richard Reinhardt** (Max-Planck Genome Centre, Max-Planck Institute for Plant Breeding Research, Cologne)
Revolutionizing transcriptome and ncRNA studies of viral and bacterial pathogens using NGS technology

Concluding Remarks

02.00 – 02.30 pm ***Coffee Break***

Satellite Symposium
Next Generation Sequencing
Organisation: Stefan Wiemann and Bernd Timmermann

Krehl Klinik Auditorium, Im Neuenheimer Feld 410

02.30 – 02.40 pm	**Welcome and Introduction** **Bernd Timmermann**
02.40 – 03.00 pm	**Stephan Wolf** (Genomics and Proteomics Core Facility, German Cancer Research Center - DKFZ, Heidelberg) *NGS at the Sequencing unit of the German Cancer Research Center*
03.00 – 03.20 pm	**Wolfgang Huber** (Computational biology and genomics, European Molecular Biology Laboratory - EMBL, Heidelberg) *Differential expression analysis of genes and exons*
03.20 – 03.40 pm	**Benedikt Brors** (Theoretical Bioinformatics, German Cancer Research Center – DKFZ, Heidelberg) *How (not) to find somatic mutations in cancer genome sequencing*
03.40 – 04.00 pm	**Saskia Biskup** (Center for Genomics and Transcriptomics – CeGaT, Tübingen) *Genome and Exome Sequencing in a diagnostic setting – Experiences from the Clarity Challenge*
04.00 – 04.10 pm	**Concluding Remarks** **Stefan Wiemann**
04.10 – 04.30 pm	***Coffee Break*** *(DKFZ Communication Center)*

Satellite Symposia Program (with speakers' biosketch)

Small RNAs

Organisation: Jürgen Haas and Jürgen Brosius

Welcome and Introduction

12.00 – 12.45 pm ***Viruses and microRNAs***
Bryan Cullen

Bryan R. Cullen obtained a B.Sc. in Biochemistry from Warwick University in the UK and a M.Sc. in Virology from the University of Birmingham before moving to the USA, where he obtained a Ph.D. in Microbiology from the University of Medicine and Dentistry of NJ. In 1987, he was recruited to Duke University Medical Center as a Howard Hughes Medical Institute Investigator and Assistant Professor. He currently holds a James B. Duke Professorship in the Department of Molecular Genetics and Microbiology and serves as Director of the Center for Virology at Duke. Bryan Cullen's interests have historically revolved around the use of viruses as genetic tools to understand aspects of the biology of the eukaryotic cell, focusing particularly on RNA-sequence mediated gene regulation. Currently, his laboratory is studying the biogenesis and function of microRNAs, and in particular virus-encoded microRNAs, and also works on human factors that act as innate inhibitors of both retrovirus infection and retrotransposon mobility.

Honors & Awards

Recipient of the 1989 Eli Lilly Molecular Biology Contact Award, Recipient of a 1993 Alexander von Humboldt Foundation Research Award, Listed as one of the 10 most cited AIDS researchers by Science (Heavy Hitters in AIDS, Vol. 260, p. 1262, 1993), Recipient of the University of Medicine and Dentistry of New Jersey Distinguished Alumni Award (2000), Awarded the distinguished professorship James B. Duke Professor of Genetics (2000), Listed as one of the world's most "Highly Cited Researchers" by the Institute for Scientific Information (2001) [http://isihighlycited.com], Appointed to the honorary position of "Visiting Professor," by the Imperial College of Science, Technology and Medicine, London, UK (2002). Awarded a Distinguished Fellowship at the Institute of Advanced Studies (IAS) at Durham University, U.K. (2007). Elected to Fellowship in the American Academy of Microbiology (2009); 16th annual Bernard Fields Memorial Lecturer at the 2011 Conference on Retroviruses and Opportunistic Infections; Elected to Fellowship in the American Association for the Advancement of Science (AAAS) (2011)

12.45 – 01.10 pm ***Host-virus interactions and microRNAs in mammals***
Sebastién Pfeffer

Degrees and diplomas

2010 HDR (Habilitation), Université de Strasbourg, France
2002 PhD in Molecular and Cellular Biology, Université Louis Pasteur, Strasbourg, France

Training and positions held

2012-present CNRS Research Director, IBMC-CNRS, Strasbourg, France
2009-2012 Group leader, IBMC-CNRS, Strasbourg, France
2005-2008 CNRS Research Associate, IBMP-CNRS, Strasbourg, France, Laboratory of Dr. Voinnet
2003-2005 Postdoctoral fellow, Rockefeller University, New York, USA. Laboratory of Dr. Tuschl
1999-2002 Graduate student, IBMP-CNRS, Strasbourg, France. Thesis advisor: Dr. K. Richards

Awards and honors

2010 Laureate of the European Research Council (ERC) starting grant program
2009 Laureate of the CNRS Claude Paoletti Scientific Prize
Laureate of the INSERM-CNRS Avenir-ATIP program
2005 Postdoctoral fellowship from The Leukemia & Lymphoma Society (USA)
2004 Exceptional project grant award from The Breast Cancer Alliance (USA)

Recent publications (40 total)

Ostermann E, et al. ***PLOS ONE*** *2012;7(8):e43744*
Tuddenham L, et al. ***J Virol*** *2012, 86(3):1638-49*
Marcinowski L, et al. ***PLOS Pathog*** *2012, 8(2): e1002510*
Suffert G, et al. ***PLOS Pathog*** *2011, 7(12): e1002405*
Buck AH, et al. ***RNA*** *2010, 16(2):307-15*

01.10 – 01.35 pm ***Analysis of Argonaute interactions in mammalian cells***

Gunter Meister

Gunter Meister is professor and chair for Biochemistry at the University of Regensburg, Germany. He obtained his PhD in 2002 from the Max-Planck-Institute of Biochemistry in Martinsried, Germany. From 2003 to 2005 he worked as postdoctoral fellow in the lab of Tom Tuschl at the Rockefeller University in New York, USA. In 2005, he started his own lab at the Max-Planck-Institute of Biochemistry as independent group leader. In 2009, he was appointed full professor and chair for Biochemistry at the University of Regensburg. His research focus is the biochemical analysis of small RNA-guided gene silencing pathways in mammals.

01.35 – 02.00 pm ***Revolutionizing transcriptome and ncRNA studies of viral and bacterial pathogens using NGS technology***

Richard Reinhardt

Richard Reinhardt studied chemistry and finished his PhD in 1978 (Röntgenstrukturanalytische Untersuchungen an homocyclischen und heterocyclischen Schwefelverbindungen (summa cum laude)). Postdoctoral Fellow (1978 to 1982) at TU/FU-Berlin (Prof. Steudel and Bradaczek), and MPI in Martinsried (Prof. Huber). Since 1982 he became senior scientist at the Max-Planck-Institute for Molecular Genetics (MPI-MG) in Berlin-Dahlem. In 1990 he became head of the scientific service- and IT-computing group of the MPI for Molecular Genetics in Berlin Dahlem. In 1994, with the appointment of Hans Lehrach and Hilger Ropers as Max-Planck directors, he set up the high throughput automation group at MPI-MG. In September 2010 he was appointed head of Max-Planck Genome Centre Cologne (MP-GC).

Concluding remarks

02.00 - 02.30 pm **Coffee Break**

Next-Generation Sequencing

Organisation: Stefan Wiemann and Bernd Timmermann

02.30 – 02.40 pm **Welcome and Introduction**
Bernd Timmermann

02.40 - 03.00 pm ***NGS at the Sequencing unit of the DKFZ***
Stephan Wolf

Stephan Wolf is a Molecular Biologist by background. As a Scientist, and later Senior Scientist, with the German Cancer Research Centre (DKFZ), Stephan has spent more than 10 years involved in Oncology research. In 2008, he was appointed as the Head of the Next Generation Sequencing Unit at the DKFZ, which is located in Heidelberg and is the largest biomedical research institute in Germany.

03.00 – 03.20 pm ***Differential expression analysis of genes and exons***
Wolfgang Huber

Wolfgang Huber is group leader and senior scientist at the European Molecular Biology Laboratory (EMBL). His research group develops computational and statistical methods to design and analyse novel experimental approaches in genetics and cell biology. He studied theoretical physics in Freiburg, did a postdoc in cheminformatics at IBM Research (San Jose, CA) and a postdoc in bioinformatics at the German Cancer Research Centre (DKFZ Heidelberg). In 2004, he joined EMBL-EBI in Cambridge and in 2009, EMBL's Genome Biology unit in Heidelberg.

03.20 - 03.40 pm ***How (not) to find somatic mutations in cancer genome sequencing***
Benedikt Brors

Studies in Chemistry, Univ. of Düsseldorf (1989-1995);
Diploma in Chemistry 1995;
PhD in Biochemistry, Univ. of Düsseldorf, 1999
Postdoctoral Researcher, Div. Theoretical Bioinformatics, DKFZ Heidelberg, 1999-2000;
Postdoctoral Researcher, Intelligent Bioinformatics Group, DKFZ, 2001-2003;
Lecturer in Bioinformatics, Univ. of Heidelberg, 2003 till present;
Group Leader Computational Oncology, Div. Theor. Bioinformatics, DKFZ; 2003 till present;
Permanent Research Staff and Group Leader, Div. Theor. Bioinformatics, DKFZ, 2009 till present.

03.40 – 04.00 pm ***Genome and Exome Sequencing in a diagnostic setting – Experiences from the Clarity Challenge***
Saskia Biskup

Saskia Biskup, MD PhD, is managing director and co-founder of CeGaT GmbH, Center for Genomics and Transcriptomics, in Tuebingen, Germany. CeGaT, founded in 2009, is specialized in next generation sequencing and data interpretation. Since its foundation CeGaT is pioneering the application of NGS in a clinical setting. In 2011, CeGaT was awarded the most successful start-up company in Germany (Deutscher Gründerpreis).
In addition Saskia Biskup is heading the Institute for Clinical Genetics at the Klinikum Stuttgart, Germany. As a Human Geneticist and Research Scientist she positions herself at the interface of diagnostics, research and clinic. During a two year scholarship sponsored by the German Research Foundation she gathered international experience at the Johns Hopkins University in Baltimore, MD, USA. Her research revolves around LRRK2, a gene involved in Parkinson's disease, and the development of new biomarkers to enable early prediction of neurodegenerative diseases.

04.00 – 04.10 pm **Concluding remarks**
Stefan Wiemann

04.10 – 04.30 pm ***Coffee Break*** *(DKFZ Communication Center)*

National Genome Research Network

Satellite Symposium I Abstracts

Small RNAs

Viruses and microRNAs

Presenting Author: Bryan Cullen

Molecular Genetics and Microbiology, Duke University Medical Center

Mammalian cells express thousands of microRNAs, which presents both opportunities and challenges for an incoming virus. Most obviously, a viral genome can encode viral microRNAs that have the potential to down-regulate genes that could limit viral replication, such as innate immune factors and pro-apoptotic proteins, and viruses may also gain advantage by inhibiting the expression of specific proteins involved in cell signaling and cell cycle regulation. Moreover, viruses could also use microRNAs to inhibit the expression of specific viral gene products, for example during latency. We now know that several pathogenic human DNA viruses, especially herpesviruses, encode multiple microRNAs and examples of each of the above activities are now known. In addition, viruses have evolved the ability to co-opt the action of specific cellular microRNAs to promote aspects of their replication cycle. For example, EBV infection of primary B cells strongly induces the cellular microRNA miR-155, which is critical for the establishment of latent viral infections in culture, while KSHV actually expresses a miR-155 analog in latently infected B cells. In this presentation, I will discuss our current understanding of the interactions of viruses with the cellular miRNA machinery, focusing on members of the herpesvirus family.

Host-virus interactions and microRNAs in mammals

Presenting Author: Sebastién Pfeffer

Architecture et Réactivité de l'ARN, Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France

Micro (mi)RNAs are small regulatory RNAs found in almost all eukaryotes. As they play key roles in fine-tuning fundamental biological processes such as anti-viral defense or cell cycle control, it is quite logical that viruses have evolved to counteract or utilize these molecules. More interestingly, some viruses, especially DNA viruses from the herpesvirus family, encode their own set of miRNAs. They can act as regulators of both host and viral genes expression, ensuring an optimal environment for the virus. Thus, miRNAs, both of cellular and viral origin, are at the very heart of host-pathogen interactions. I will discuss the impact of viral infection on host miRNA expression, and present some evidence that viruses can sometimes counteract deleterious effects mediated by cellular miRNAs. I will also present an overview of virally encoded miRNAs and their expression during infection, followed by examples of some biological roles of these viral regulators. Although we are only starting to fully grasp the importance of viral miRNAs, it is evident that evolutionary distant viruses have evolved to express miRNAs towards convergent functions. Finally, we will also see that getting a precise insight into the relative importance of cellular and viral miRNAs during infection is much harder than previously thought.

Analysis of Argonaute interactions in mammalian cells

Presenting Author: Gunter Meister

Chair of biochemistry, University of Regensburg, Germany

Small RNAs such as short interfering RNAs (siRNAs) or microRNAs (miRNAs) are more and more emerging as small molecules that have key-regulatory functions. Small RNAs are found in all higher eukaryotes and play important roles in cellular processes as diverse as development, stress response or transposon silencing. Furthermore, miRNAs are also found in some viruses including the Epstein-Barr-Virus (EBV) or Kaposi's Sarcoma-associated Herpesvirus (KSHV). Members of the Argonaute protein family are mediators of gene silencing and bind to all small regulatory RNA classes. Ago proteins are characterized by PAZ, MID and PIWI domains and facilitate target RNA cleavage in RNA interference (RNAi) or miRNA-guided repression of gene expression. These proteins interact with many different cellular proteins either direct or via common RNA molecules and form large regulatory networks. For a detailed understanding of Ago protein function, it is important to identify such cellular binding partners. Here, we report the analysis of Ago protein interactions in miRNA-containing and miRNA-depleted cells. Using Stable Isotope Labeling in Cell Culture (SILAC) in conjunction with Dicer knock out mouse embryonic fibroblasts (MEFs), we identify proteins that interact with Ago2 in the presence or the absence of miRNAs. In contrast to our current view, we find that Ago-mRNA interactions can also take place in the absence of miRNAs. Furthermore, we compare wt and EBV-infected cells in order to identify EBV proteins that might influence Ago2 function. Our proteomics approach provides a rich resource for further functional studies on the cellular roles of Ago proteins.

Revolutionizing transcriptome and ncRNA studies of viral and bacterial pathogens using NGS technology

Presenting Author: Richard Reinhardt
Cynthia M. Sharma, Jörg Vogel, Jens Gruber (RNomics consortium)

Max-Planck Genome Centre, Max-Planck Institute for Plant Breeding Research, Cologne

Next-generation sequencing (NGS) has been revolutionizing many medical and biological applications and its contribution to life science has outcompeted even the application of PCR-based methods in the past twenty years in the medical area. For example, Illumina RNA deep sequencing (RNA-Seq) is nowadays used as a widespread tool to sequence millions of cDNAs which allows for a real deep insight into the transcriptome structure of cells. Furthermore, RNA-seq is now also used for transcriptome profiling instead of microarrays, especially since the costs for NGS methods are steadily decreasing and the ongoing development of new instruments will also allow for new application such as single cell sequencing within the next future.
Besides some technical aspects of NGS technology including library preparation, we will present our results of several RNA-seq projects of viral1 and bacterial2 pathogens within the “RNomics of Infection” consortium.
The study of small npcRNAs after in-vitro-infection with HIV-1, characterized 31 µRNAs with modified expression after infection, including miR-29, which is expected to play a role in the transcriptional regulation of HIV-1
Furthermore, we have developed a novel differential RNA-seq (dRNA-seq) approach selective for the sequencing of primary transcriptomes which allowed us to define a genome-wide map of transcriptional start sites in the human pathogen, Helicobacter pylori2. Moreover, our approach revealed a massive antisense transcription and an unexpected number of more than 60 small RNAs. We have successfully applied this approach to a wider range of pro- and eukaryotes, including additional human pathogens such as Chlamydia 4,5. Furthermore, we have successfully combined co-immunoprecipitation with RNA-seq to analyze RNA-protein complexes e.g. in Helicobacter6. Overall, our RNA-seq based methods give new insights into transcriptome structure and ncRNA repertoires of diverse pathogens and will increase our knowledge on genome organization as well as RNA-based regulation in a wider range of organisms. Furthermore, the ongoing development of NGS methods will allow to study transcriptomes of host and pathogen in parallel during the time-course of infection.

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3) Albrecht M, Sharma CM, Dittrich MT, Müller T, Reinhardt R, Vogel J, Rudel. The transcriptional landscape of Chlamydia pneumoniae. T. Genome Biol. 2011 Oct 11;12(10):R98.
4) Albrecht M, Sharma CM, Reinhardt R, Vogel J, Rudel T. Deep sequencing-based discovery of the Chlamydia trachomatis transcriptome. Nucleic Acids Res. 2010 Jan;38(3):868-77.
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National Genome Research Network

Satellite Symposium II Abstracts

Next-Generation Sequencing

NGS at the Sequencing unit of the German Cancer Research Center

Presenting Author: Stephan Wolf

Genomics and Proteomics Core Facility, German Cancer Research Center - DKFZ, Heidelberg

NGS can no longer be imagined as anything else but an integral part of molecular biology and medical research. At the DKFZ Sequencing unit, which was set up in 2010, the scientific work groups at DKFZ have access to the most technically advanced systems as well as the expertise of a total of 20 scientists, bioinformatics experts, engineers and technicians. Nine Illumina HiSeq 2000, a HiSeq 2500, a Roche 454 FLX and two Illumina MiSeq Systems are available here, making the unit one of the largest in Europe.
Whole genome sequencing, RNA sequencing, smallRNA sequencing, whole genome bisulfite sequencing, ChIP sequencing and exome enrichment/sequencing are the standard applications provided by the unit.
The sequencing unit sees itself not just as a service provider and enabler for established research approaches and protocols, but also endeavours, together with the different research groups, to develop and integrate innovative ideas and new technologies.
The DKFZ sequencing unit makes substantial contributions to the international ICGC PedBrain and prostate cancer projects and is the sequencing basis for the new Heidelberg Center for Personalised Oncology (HIPO) as well as the Precision Oncology Program (POP) and is one of the sequencing platforms for the German Consortium for Translational Cancer Research (DKTK).
During my talk I will introduce the NGS work at the German Cancer Research Center, provide insights into technical difficulties when running NGS in a high throughput service environment and present some recent improvements in NGS workflows and protocols.

Differential expression analysis of genes and exons

Presenting Author: Wolfgang Huber

Computational biology and genomics, European Molecular Biology Laboratory - EMBL, Heidelberg

RNA-Seq is a powerful tool for the study of alternative splicing and other forms of alternative isoform expression. Understanding the regulation of these processes requires sensitive and specific detection of differential isoform abundance in comparisons between conditions, cell types or tissues. I will present DEXSeq, a statistical method to test for differential exon usage in RNA-Seq data. DEXSeq employs
generalized linear models and an error model adapted to RNA-Seq data, providing reliable control of false discoveries by taking biological variation into account. DEXSeq detects genes, and in many cases specific exons, that are subject to differential exon usage with high sensitivity. The versatility of DEXSeq is shown by applying it to several data sets. The method facilitates the study of regulation and function of alternative exon usage on a genome-wide scale. An implementation of DEXSeq is available as an R/Bioconductor package.

How (not) to find somatic mutations in cancer genome sequencing

Presenting Author: Benedikt Brors

Theoretical Bioinformatics, German Cancer Research Center - DKFZ, Heidelberg

Recent developments in sequencing technology have made it possible to sequence entire cancer genomes with high coverage to uncover somatic genetic alterations in cancer. However, the situation is sometimes complicated by varying degrees of tumor cellularity, polyploidy in tumor genomes, and presence of tumor subpolulations. In addition, sequencing technology influences sensitivity and specificity by bias with respect to local GC content, by systematic sequencing errors, and by mappability of genomic regions due to presence of repetitive elements. I will describe the power and limits of current cancer genome sequencing endeavours in this context and also show the impact on our understanding of cancer as an acquired genetic disease.

Genome and Exome Sequencing in a diagnostic setting – Experiences from the Clarity Challenge

Presenting Author: Saskia Biskup

Center for Genomics and Transcriptomics – CeGaT, Tübingen

Rapid developments in next-generation sequencing (NGS) technologies have made genome and whole exome sequencing a very efficient approach to elucidate the genetic basis of diseases with so far unknown etiology. NGS is mostly applied in a research setting, but recently target enrichment of known disease genes and also whole exome sequencing is increasingly being employed as a diagnostic tool in a clinical setting. Disorders characterized by significant genetic and phenotypic heterogeneity, for example, neurodegenerative diseases, neuromuscular diseases, epilepsies, hereditary deafness, hereditary vision loss, cancer and many more are most suitable for a panel of genes based approach as it is sometimes very challenging to prioritize candidate genes and conventional sequencing does not allow comprehensive screening due to high sequencing costs. There is no doubt that now available diagnostic panels for heterogeneous diseases completely changed genetic testing in a clinical setting. The question rather is whether whole genome or whole exome sequencing are ready for prime-time clinical applications. In January this year, the Children's Hospital in Boston put out the call for submissions, asking participants to help determine the unknown genetic cause of illness in three families. The goal of the so called CLARITY Challenge (Children's Leadership Award for the Reliable Interpretation and appropriate Transmission of Your genomic information) was to identify best methods and practices for the analysis, interpretation and reporting of sequence data, to provide the most meaningful results to clinicians, patients and families. The ultimate aim of the competition was to shed light on how data from whole genome sequences can be made most useful in a clinical setting. Here, I will be presenting the results and will be summarizing the current status of next generation sequencing in a diagnostic setting, the technical challenges facing it, and its use as a routine diagnostic tool.

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Main Program

Program (with speakers' biosketch)

Tuesday, December 11, 2012

Satellite Symposia - Krehl Klinik Auditorium, Im Neuenheimer Feld 410

12.00 – 02.00 pm **Satellite Symposium**: Small RNAs (Org. Jürgen Haas and Jürgen Brosius)

02.00 – 02.30 pm **Coffee Break**

02.30 – 04.00 pm **Satellite Symposium**: Next-Generation Sequencing (Org. Stefan Wiemann and Bernd Timmermann)

04.00 – 04.30 pm **Coffee Break** (DKFZ Communication Center)

Welcome - DKFZ Communication Center, Main Auditorium

04.30 – 05.00 pm **Wolfgang Wurst**, Helmholtz Zentrum Munich, Germany, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

N.N., Federal Ministry of Education and Research, Germany

Christof von Kalle, National Center for Tumor Diseases (NCT)/ German Cancer Research Center (DKFZ), Heidelberg, Germany

Symposium I: International Projects in the Program of Medical Genome Research

05.00 – 05.30 pm ***International Cancer Genome Consortium (ICGC)***
Translation of the Cancer Genome
Lynda Chin

Dr. Lynda Chin received her M.D. from the Albert Einstein College of Medicine in 1993, and is a board-certified dermatologist. She conducted her clinical and scientific training at Columbia Presbyterian Medical Center and the Albert Einstein College of Medicine where she was appointed Chief Resident of Dermatology. For the first 14 years of her independent career, she was a member of the Dana-Farber Cancer Institute and Harvard Medical School communities where she was Professor of Dermatology at the Harvard Medical School, member of the Department of Medical Oncology at Dana-Farber Cancer Institute, and a Senior Associate Member of the Broad Institute of MIT and Harvard. Dr. Chin was the founding Scientific Director of the Belfer Institute for Applied Cancer Science at the Dana-Farber Cancer Institute. In 2011, Dr. Chin joined The University of Texas MD Anderson Cancer Center where she launched and chairs the Department of Genomic Medicine, in addition to serving as the Scientific Director of the Institute for Applied Cancer Science. Dr. Chin is actively involved in The Cancer Genome Atlas (TCGA). In addition to being PI and co-PI, respectively, in two TCGA centers (the Genome Data Analysis Center at the Broad and the Genome Characterization Center at HMS), she also serves on its Executive Subcommittee and chairs the Analysis Working Groups in GBM and Melanoma. She is also a member of the Scientific Steering Committee of the International Cancer Genome Consortium (ICGC). Dr. Chin co-founded AVEO Pharmaceuticals in 2002, a cancer biotechnology company that emphasizes cancer biology and genetics to identify new cancer targets with tumor maintenance roles. She also founded Metamark Genetic, a cancer diagnostic company that will develop function-based prognostic determinants that can guide customized management of early-staged cancer patients including melanoma and prostate cancer. Dr. Chin has three young children, ages 11, 10 and 8. Most recently she was elected to the Institute of Medicine of National Academies (IOM).

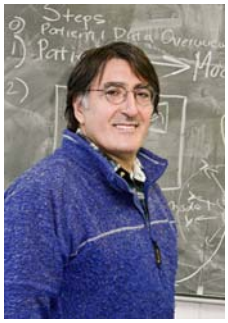
05.30 – 06.00 pm ***International Cancer Genome Consortium (ICGC)***
Novel findings in pediatric brain tumors revealed by high resolution genome, methylome and transcriptome analyses
Peter Lichter

Professor Peter Lichter studied biology at the University of Heidelberg in Germany, where he accomplished his Ph.D. in 1986. He then was a postdoctoral scientist in the laboratory of Prof. D.C. Ward in the Department of Genetics at the School of Medicine in Yale University, New Haven, USA. 1990 he returned to Heidelberg and became head of the project group "Organization of complex genomes". Since 1992 Prof. Lichter is the head of the division "Molecular Genetics" at the DKFZ in Heidelberg. In 2000 he became full Professor at the Faculty of Medicine, University of Heidelberg. Prof. Lichter took up the post of Interim Director of the Management Board of the DKFZ in 2003. The main research topics of Prof. Lichter include the pathomechanisms of tumor development, tumor markers, molecular profiling of tumor cells and genome organization, and gene function. He has received several awards for his work, such as the German Cancer Award (2002), the Award of Deutsche Krebshilfe (2003) and the award of the European Society of Human Genetics (2012). Prof. Lichter is coordinating the PedBrain Tumor Project in the International Cancer Genome Consortium (ICGC) since December 2009. Moreover he is member of the project committee of the National Genome Research Network (NGFN-Plus) and coordinator of the Integrated Genome Research Network "Brain tumor" within NGFN-Plus. He was member of the DFG Hinterzartener Kreis, the Scientific Program Committee of the European Society of Human Genetics, the Science Council and is moreover member of EMBO, and Leopoldina.

06.00 – 06.30 pm ***International Cancer Genome Consortium (ICGC)***
Genome Alterations in Human Lung Cancers
Matthew Meyerson

Matthew Meyerson is a leader in the field of cancer genomics with a focus on lung cancer. He serves as Professor of Pathology at Dana-Farber Cancer Institute and Harvard Medical School, and a Senior Associate Member of the Broad Institute. Together with Drs. Bill Sellers, Bruce Johnson and Pasi Janne, the Meyerson group identified somatic mutations in the epidermal growth factor gene, EGFR, in lung adenocarcinomas, that predict response to the EGFR kinase inhibitors, gefitinib and erlotinib. The Meyerson group and collaborators have also discovered other activated kinase genes in cancer, including JAK2 in polycythemia vera, FGFR2 in endometrial cancer, ALK in neuroblastoma, and DDR2 and FGFR1 in squamous cell lung cancer. The laboratory has pioneered technical and computational approaches for cancer genome research, including methods for copy number determination with single nucleotide polymorphism (SNP) arrays, leading to identification of oncogenes including NKX2-1, SOX2, and MCL1. In addition, the Meyerson group performed the first next-generation sequencing analysis of cancer DNA and developed the computational subtraction approach to discover novel disease-causing microbes. Dr. Meyerson plays a leading role in "The Cancer Genome Atlas" (TCGA), as principal investigator of the Genome Characterization Center at the Broad Institute, focused on copy number and structural alterations. He co-chairs the lung cancer working group with Drs. Steve Baylin and Ramaswamy Govindan. Dr. Meyerson and Dr. Bill Hahn direct the Center for Cancer Genome Discovery at Dana-Farber Cancer Institute. Dr. Meyerson received his M.D. from Harvard Medical School and his Ph.D. from Harvard University. He served as a resident in Clinical Pathology at Massachusetts General Hospital and a post-doctoral fellow at the Whitehead Institute with Dr. Robert Weinberg. Among other prizes, Dr. Meyerson has been awarded the Paul Marks Prize in Cancer Research from Memorial Sloan-Kettering Cancer Center and the Team Science Award from the American Association for Cancer Research.

06.30 – 07.00 pm ***1000 Genomes Project – An integrated map of genetic variation***
Hans Lehrach



Hans Lehrach studied Chemistry at the University of Vienna and accomplished his Ph.D. in 1974 at the MPIs for Experimental Medicine and for Biophysical Chemistry, Göttingen. He was a research fellow at Harvard University. After his return to Europe he became a research group leader at the EMBL, Heidelberg, and later on head of the Department of Genome Analysis at the ICRF. Since 1994, Hans Lehrach is Director at the MPI for Molecular Genetics and Professor at the Free University Berlin. Hans Lehrach was a speaker of the DHGP and a member of the project committee of the NGFN. Moreover, he is a member of the European Molecular Biology Organization (EMBO), of the scientific advisory board of the Archon Genomic X PRIZE, as well as of Editorial boards of several scientific journals. He is member of the Scientific Committee of the European Science Foundation (ESF), Medical Sciences, Personalised Medicine for the European Citizen. He participated in the Human Genome Project, is partner in two German ICGC projects and partner in the European IHEC project BLUEPRINT, he is steering committee member of the 1000 Genomes Project, leader of the managing entity of IMI OncoTrack project and coordinator of the European FET Flagship initiative on personalised medicine IT Future of Medicine.

07.00 pm **Supper**

Wednesday, December 12, 2012
Symposium II: International Projects in Genome Research

09.00 – 9.30 am ***Identification of Complex Disease Genes Using Whole Genome Sequencing in Large Pedigrees***
John Blangero

John Blangero is a Scientist at Texas Biomedical Research Institute in San Antonio where he has worked for the past 26 years after obtaining his PhD in human population genetics at Case Western Reserve University. He is a pioneer in the area of statistical genetics of complex diseases and has published over 500 articles whilst controlling competitive grant funding in excess of $6.5M US per annum. Blangero has been a devoted advocate of large pedigree studies for the identification of complex disease-related genes and for the likely importance of rare variants in normal quantitative variation. He is in charge of the development and maintenance of the SOLAR software package for statistical genetics that is now used by more than 5000 investigators world-wide. Currently, he directs the activities of one the world's largest super computers, MEDUSA, dedicated to human genomics. In his spare time, he plays keyboards and sings in a Southern blues band and records under the name “The Sun King Reborn”.

09.30 – 10.00 am ***De novo diagnostics in patients with intellectual disability***
Joris A. Veltman

Dr. Joris A. Veltman is Associate Professor at the Department of Human Genetics, Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. His work focuses on the identification and interpretation of genomic variation, with a particular interest in the role of rare *de novo* mutations in severe neurodevelopmental and psychiatric diseases (ERC project DENOVO 2011). He studies the genomes of these patients using high-resolution genomic microarrays as well as next generation sequencing technology and combines laboratory experiments with novel bioinformatic approaches. Dr. Veltman is also actively involved in the implementation of these novel genomics approaches in routine clinical diagnosis.

10.00 – 10.30 am ***Alzheimer's Disease Genetics Consortium (ADGC)***
Neurodegenerative disease genetics; GWAS, exomes and beyond
Gerard D. Schellenberg

Dr. Schellenberg received his Ph.D. in biochemistry from the University of California, Riverside in 1978. Following a postdoc in Genetics and Neurology at the University of Washington, he was appointed Research Assistant Professor in Neurology in 1983 and rose to the rank of Research Professor in 1995. Dr. Schellenberg moved to the University of Pennsylvania in 2008 where he is presently a Professor in the Department of Pathology and Laboratory Medicine. For the past 22 years, Dr. Schellenberg has worked on the genetics of Alzheimer's disease, starting with ground-breaking research on early-onset familial Alzheimer's disease followed by work on late-onset dementia which is where much of his current effort is focused. Dr. Schellenberg has received several awards for his research on Alzheimer's disease. He is founder and head of the Alzheimer's Disease Genetics Consortium (ADGC) supported by the National Institute on Aging. Furthermore, Dr. Schellenberg worked on the genetics of aging and his group identified the gene for Werner's Syndrome, a premature aging syndrome. He also studies the molecular genetics of other neurodegenerative disorders related to Alzheimer's disease with a focus on frontotemporal dementia, Guam amyotrophic lateral sclerosis/parkinsonism dementia complex and progressive supranuclear palsy. This work includes using invertebrate and vertebrate model organisms to study tauopathies. Dr. Schellenberg is also working on unraveling the genetics of autism, a complex neurodevelopmental disorder. He participates in the Autism Genome Project consortium. His current efforts focus on deep sequencing of genes suspected of being involved in autism risk.

10.30 – 11.00 am ***Coffee Break***

11.00 – 11.30 am ***International Human Epigenome Consortium (IHEC)***
The Cancer Epigenome
Peter Jones

Peter Jones was born in South Africa, raised and attended school in Rhodesia (now Zimbabwe), and received his Ph.D. from the University of London in 1973. He is currently a Distinguished Professor of Urology and Biochemistry & Molecular Biology and the Sawyer Chair in Cancer Research at the Keck School of Medicine USC. He was the Director of the University of Southern California Norris Comprehensive Cancer Center from 1993 to 2011. He is known for his studies on the molecular biology cancer and of basic mechanisms of DNA methylation and its role in cancer and differentiation. His laboratory discovered the effects of 5-azacytidine on DNA methylation and linked this process to the activation of silenced genes. He joined the USC in 1977, attaining the rank of Professor in 1985, and became Director of the Cancer Center in 1993. He is the author of more than 300 journal publications and book chapters, and serves on several national and international committees, panels, and editorial boards. He is the past President of the American Association for Cancer Research. He has received several honors, including the Outstanding Investigator Grant from the National Cancer Institute, shared the Kirk A. Landon Award for Basic Cancer Research from the AACR in 2009, and the Medal of Honor from the American Cancer Society in 2011 with Stephen Baylin.

11.30 – 12.00 pm ***International Human Epigenome Consortium (IHEC)***
Control of DNA-methylation in development
Jörn Walter

Jörn Walter was born in Germany and received his PhD from the Free University in Berlin in 1990. Following a postdoc at the BBSRC in Cambridge, UK he became a group leader at the MPI for Molecular Genetics in Berlin. Since 2000 he is the chair of genetics at the Saarland University in Saarbrücken. He is known for his work on mechanisms of DNA-methylation particularly in the field of genomic imprinting, epigenomics and reprogramming. His laboratory discovered mechanisms of DNA-demethylation in mammals.
He is an author of some 80 journal publications and book chapters and serves on several national and international commitees, national panels and editorial boards. He coordinated several international (EU, HFSP) and national (DFG, BMBF) research programs on epigenetics. He currently coordinates the BMBF funded research initiative DEEP (= Deutsches Epigenom Programm) the german contribution to the International Epigenome Consortium (IHEC).

12.00 – 02.00 pm ***Lunch Break and Poster Session I***

Company Satellite Sessions *(Conference Room K1)*

12.10 – 12.40 pm **Maik Pruess, Affymetrix UK Ltd**
From basic research to clinical samples. When are microarrays the right tool for the job?

12.45 – 01.15 pm **Hannes Arnold,Caliper a PerkinElmer Company**
Automation of NGS Sample Preparation: From Benchtop NGS to Genome Centers

01.20 – 01.50 pm **Luc Smink, Illumina UK Ltd**
Illumina's Genomics Research Portfolio: From Whole Genomes to Targeted approaches

01.55 – 02.25 pm **Pia Scheu, Bio-Rad Laboratories GmbH**
Droplet Digital PCR: Molecular Biology in High Resolution

Symposium II: International Projects in Genome Research

02.00 – 02.30 pm ***International Knockout Mouse Consortium / EUCOMM: Resource to functionally validate all genes***
Wolfgang Wurst

Prof. Dr. Wurst is Director of the *Institute of Developmental Genetics* at the Helmholtz Zentrum München in Neuherberg and Professor at the *Technical University Munich*. Since September 2009 he has been also Cooperation Partner of the *DZNE (Deutsches Zentrum für Neurodegenerative Erkrankungen in der Helmholtz-Gemeinschaft),* Site Munich, Germany. Previous positions: Group leader of the group Molecular Neurogenetics at *the Max-Planck-Institute of Psychiatry* in Munich and Junior Research Group Leader at the Department of Mammalian Genetics, *GSF Research Center*, Neuherberg/Munich. Prof. Dr. Wurst studied Biology/Chemistry at the *University of Freiburg*, Germany, and obtained his PhD at the University of Göttingen, Germany. He has longstanding expertise in neural development and molecular neurogenetics. He provides important advice in the establishment of animal models of depression disorders and generates knock-out animals. He is coordinating large international consortiums with respect to generating animal models for human diseases. Currently he is one of the Speakers of the Project Committee of the National Genome Research Network (NGFNplus). Further Research Interests: Molecular genetics, embryology, molecular neurogenetics, neural development, neural pattern formation, behaviour, neurological and psychiatric disease models, gene function analysis in vivo (gene trapping, gene targeting, RNA interference).

02.30 – 03.00 pm ***EUMODIC and INFRAFRONTIER - Creating a comprehensive encyclopedia of mammalian gene function***
Martin Hrabě de Angelis

Prof. Dr. Martin Hrabě de Angelis studied biology at the Philipps University in Marburg and received his PhD in 1994. He worked as postdoctoral fellow from 1994-97 at the Jackson Laboratory in Bar Harbor/USA studying the Delta/Notch pathway and mouse mutant lines with impaired somitogenesis. In 2000 he was recruited as director of the Institute of Experimental Genetics at the Helmholtz Zentrum München (formerly GSF). Hrabě de Angelis is director of the European Mouse Mutant Archive in Monterotondo/Rome Italy. In 2001 he founded the German Mouse Clinic (GMC) for systemic analysis of human diseases. He is member of the project committee of the National Genome Research Network (NGFN). Prof. Hrabě de Angelis published over 250 original works and is author of several specialist books.
Moreover, he is one of the founders and members of the steering committee of the German Center for Diabetes Research DZD e.V. which has been established in 2009.

03.00 – 03.30 pm ***High resolution, quantitative mass spectrometry combines proteomics and genomics***
Matthias Mann

Matthias Mann studied physics and mathematics at Göttingen University in Germany and obtained his Ph.D. in chemical engineering at Yale University. Here he was decisively involved in the development of electrospray ionization, which has become a key technology of the life sciences. As a post-doctoral fellow and later as a professor for bioinformatics at the University of Southern Denmark in Odense, he developed, amongst others techniques, the first bioinformatic search algorithms for peptide fragmentation data and SILAC, a new method of quantitative proteomics and a breakthrough in the mapping of protein interactions. Since 2005 Matthias Mann has been director at the Max-Planck Institute of Biochemistry in Martinsried near Munich, Germany and from 2009 also the department head of proteomics at the Novo Nordisk Foundation Center for protein research in Copenhagen, Denmark. Dr. Mann has authored and co-authored more than 500 publications with a total citation count of more than 70,000, which according to the Institute for Scientific Information, makes him one of the most highly cited researchers worldwide. He has been elected member of the European Molecular Biology Organization as well as the Royal Danish Academy of Arts and Sciences and also to a visiting professorship at Harvard Medical School. He has received two honorary degrees from Utrecht University and the University of Dundee, respectively.
In 2012 he has been awarded the Leibniz Prize from the German Research Foundation, the Ernst Schering Prize, the Louis-Jeantet Foundation Prize for Medicine and the Körber European Science Prize.

03.30 – 04.00 pm ***Coffee Break***

Symposium III: Genomics of Common Disease I

04.00 – 04.15 pm ***Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: An exome sequencing study***
André Reis

André Reis studied medicine and in 1995 received his faculty affiliation from Charité, Humboldt University Berlin. At the Max-Delbrück-Centre he founded and led the “Gene Mapping Centre” (1995-2000) and in 1998 was appointed associate professor at Charité. Since 2000 he is full professor and director of the Institute of Human Genetics at the University of Erlangen-Nuremberg. He served as member of the steering committee of the German Human Genome Project (DHGP) (2001-2004) and as president of the German Society of Human Genetics (GfH) (2008-2012). Research in his laboratory focuses on elucidation of the molecular basis of both Mendelian and complex traits i.e. psoriasis, glaucoma and intellectual disability. In NGFNplus he coordinated the German Mental Retardation Network (MRNET), a network aiming at the understanding of the molecular basis of intellectual disability (mental retardation).

04.15 – 04.30 pm ***Pre-, peri- and postnatal stress in human and non-human offspring: A convergent approach to study genetic and epigenetic impact on depression***
Vanessa Nieratschker

Vanessa Nieratschker did her PhD in Genetics and Neurobiology at the Julius-Maximilians-University of Würzburg. For her thesis, she studied a kinase in Drosophila melanogaster which interacts with the synaptic protein "Bruchpilot", a homolog to vertebrate ERC/ELKS/CAST. Following her PhD she joined the Department of Genetic Epidemiology in Psychiatry at the Central Institute of Mental Health as Head of the Molecular Genetics Laboratory. The group she is working in studies the genetic and epigenetic basis of mental disorders including stress related disorders and depression. One exiting project she is involved in is POSEIDON („Pre-, peri- and postnatal stress in human and non-human offspring: a translational approach to study epigenetic impact on depression"). Vanessa Nieratschker will be presenting the first genetic and epigenetic results at the 5th Annual Meeting of NGFN-Plus and NGFN-Transfer. As of January 2013, she will start as a Juniorprofessor for Molecular Psychiatry at the University of Tübingen.

04.30 – 04.45 pm ***Host-microbe interactions shape genetic risk for inflammatory bowel disease***
Andre Franke

Andre Franke is leading the genetics and bioinformatics group within the institute of Clinical Molecular Biology (ICMB) of which he is one of the directors. He is also in charge of the high-throughput facilities at the ICMB. In August 2008 Dr. Franke became Juniorprofessor within the DFG excellence cluster "Inflammation at Interfaces" and in April 2010 he received the W2 Peter Hans-Hofschneider endowment professorship of the foundation for Experimental Biomedicine.
Dr. Franke's main interests are the development and establishment of novel high-throughput genetic/genomic technologies, the inherent bioinformatic integration and the application of both to identify the genetic and epigenetic causes of chronic inflammatory plus other complex diseases like Crohn's disease and psoriasis. Having worked primarily on genome-wide association studies for the last years, Dr. Franke's research agenda currently focuses on other high-throughput screening agendas such as microRNA, microbiome and integrated approaches. Novel foci are also the establishment of a clinical data warehouse and management system and leading the DFG Research Training Group "Genes, Environment and Inflammation"

04.45 – 05.00 pm ***Mapping the functional epigenome of Ulcerative Colitis in monozygotic twins***
Robert Häsler

After studying biology at the Eberhard-Karls-University of Tübingen and at the University of Konstanz, Robert Häsler obtained his Ph.D. at the Department of Dermatology, University of Kiel in 2001, where he was working on inflammatory host-pathogen interactions under the supervision of Prof. Jens Michael Schröder. In 2001 he joined the research group of Prof. Stefan Schreiber at the 1st Medical Department of the University-Clinic Schleswig-Holstein, Kiel where he worked on transcriptome analysis in inflammatory bowel diseases. Since its foundation in 2004, he is a member of the Institute of Clinical Molecular Biology at Christian-Albrechts-University of Kiel, working on host-microbiome interactions and functional genomics in the context of inflammatory bowel diseases. He contributed to the identification of various molecular mechanisms and pathways involved in the susceptibility, manifestation and progression of ulcerative colitis and Crohn's disease.

05.00 – 05.30 pm ***Coffee Break***

05.30 – 05.45 pm ***Childhood ADHD and obesity: Evidence for a common genetic link***
Anke Hinney

Anke Hinney, Prof., PhD began her professorship in Molecular Genetics of Obesity and Eating Disorders at the University of Duisburg-Essen in October 2012. Previously, she was head of the research division, 'Molecular Genetics', in the Department of Child and Adolescent Psychiatry at the same university. From 1995 – 2004, she was head of the molecular genetic laboratory of a DFG-clinical research group in the Department of Child and Adolescent Psychiatry at Philipps-University of Marburg. She received her PhD from the Institute of Anthropology and Human Genetics at the University of Tübingen and completed her PostDoc at the University of Düsseldorf. Extensive expertise in the field of molecular genetic studies in complex disorders with a focus on childhood and adolescence (more than 170 peer reviewed original articles and reviews; several grants BMBF, NGFN, EU, DFG). Supervision of MD and PhD students.

05.45 – 06.00 pm ***An integrated catalogue of genome, methylome and gene expression variations in colon cancer***
Ralf Herwig

Ralf Herwig studied Physics and Mathematics at the TU and FU Berlin and did his PhD in bioinformatics on methods for analyzing gene expression data. Since 2001 he is group leader at the Max Planck Institute for molecular Genetics in Berlin. The bioinformatics group develops statistical methods and computational tools and resources for the analysis and interpretation of biological data predominantly in the domain of genome research. Furthermore, the focus of the group is the analysis of genomic data with molecular networks. The group is well integrated in the medical genome research and systems biology research communities and is interested in applying genome analysis for improving personalized therapies in different disease domains, in particular cancer. Ralf Herwig is member of worldwide projects such as the 1000 Genomes Project and the International Cancer Genome Consortium that apply high-throughput sequencing technologies for studying human genomes.

Evening Lecture:

06.00 – 07.00 pm ***Evolutionary Medicine and Molecular Medicine: Synergistic Siblings***
Randolph Nesse

Randolph M. Nesse M.D. is a founder of the field of evolutionary medicine who continues to lead efforts to use evolutionary principles to solve problems in medicine and public health. He collaborated with the eminent evolutionary biologist George Williams to author several seminal publications including Why We Get Sick: The New Science of Darwinian Medicine. He edits The Evolution and Medicine Review and serves as President of the Foundation for Evolution, Medicine, and Public Health. His current research focuses on how natural selection shaped the mechanisms that regulate defenses, including emotions, according to the principles of signal detection theory. A secondary focus is on how social selection can shape traits otherwise often attributed to group selection. His most recent three articles are about standards of evidence in evolutionary medicine, evolutionary molecular medicine, and evolutionary applications in cancer biology. He is based at the University of Michigan where he is Professor of Psychiatry, Professor of Psychology, Research Professor at the Institute of Social Research, and Director of the Evolution and Human Adaptation Program. His website is at http://nesse.us

07.00 – 10.00 pm ***Get-Together (Wine, Cheese, Live Music)***

Thursday, December 13, 2012

Symposium IV: Genomics of Common Disease II

09.00 – 09.30 am **Keynote Presentation:**
Innovations in translational research – Successes of NGFN
Hugo A. Katus

Professor Hugo A. Katus studied medicine at the University of Heidelberg in Germany, where he accomplished his M.D.-Degree in 1976. From 1978 to 1980 he worked as a research fellow at the Massachusetts General Hospital, Harvard Medical School, in Boston. In 1986 he then became a senior physician at the department of Internal Medicine and Cardiology at Heidelberg University Hospital. 1988 he became associate professor of Internal Medicine at the University of Heidelberg where he took up the post of vice director for the department of Internal Medicine III (Cardiology, Angiology, Pulmonology) and became in 1992 director of the Cardiac Catheterization Unit. In 1996 he also became professor of Internal Medicine and head of Internal Medicine Cardiology at the University of Lübeck, Germany. Since 2002 he is professor of Internal Medicine and director of the Department of Internal Medicine III at the University of Heidelberg. Prof. Katus has received numerous awards for his work in the field of cardiovascular medicine. Moreover Prof. Katus is member of the project committee of the National Genome Research Network (NGFN-Plus), and was 2008-2010 speaker thereof. He is coordinator of the Integrated Genome Research Network "Genetics of Heart Failure" within NGFN-Plus. Besides being President of the Academy of the German Society of Cardiology, he also is an editor of the official scientific journal of the German cardiac society "Clinical Research in Cardiology". Prof. Katus is member of the International Society for Heart Research, the American Heart Association, the German Chapter of ACC, the German cardiac society, as well as a fellow of the European Society of Cardiology and of the American College of Cardiology. Prof. Katus has published 780 original articles.

09.30 – 09.45 am ***Association of X-chromosomal variants with coronary heart disease: Results from a meta-analysis***
Christina Loley

09.45 – 10.00 am ***Toward a novel genomics-based taxonomy of lung cancer: The Clinical Lung Cancer Genome Project***
Martin Peifer

Martin Peifer has studied physics at the Albert-Ludwigs University of Freiburg where he received his Ph.D. degree. Following postdoctoral fellowships at the University of Graz and the Max-Planck Institute for Neurological Research in Cologne he is currently holding a postdoc position at the Institute of Translational Medicine at the University of Cologne. His main research focus is the analysis of high-throughput cancer genomics data with the aim to identify pathologically relevant genome alterations that might lead to new strategies of cancer treatment.

10.00 – 10.15 am ***Aberrant self-renewal and quiescence contribute to Glioblastoma aggressiveness***
Christel Herold-Mende

2012	Professor for Experimental Neurosurgery
2010	Professorship, Heidelberg University
2006-present	Head of the Div. Neurosurgical Research (Dpt. Neurosurgery) Head of the Molecular Cell Biology Group (ENT Dpt.)
2006	Habilitation
1996-2006	Head of the Molecular Laboratory (Dpt. Neurosurgery) and of the Molecular Cell Biology Group (ENT-Dpt.)at Heidelberg University
1996	Postdoc with Dr. R. Gerhards, Ruhruniversität Bochum, Dpt. Applied, Experimental and Interdisciplinary Oncology
1994-1995	Research associate with Prof. Dr. P. Bannasch; DKFZ, Div. Cytopathology
1991-1995	Graduate student with Prof. Dr. U. Schairer and Dr. F.X. Bosch; ZMBH and ENT-Department, Heidelberg University, Ph.D.
1981-1990	Sports and Biology; Biology and Chemistry; Heidelberg University.

Awards, Honors

2004	Sybille Assmus Price
2005	Clinical Science Award 2004, German Society for Immunotherapy
2005	HGF Ideenwettbewerb „Stem cells and glioma“
2010	EANO IX Posteraward in the field of “Immunology and Immunotherapy”

10.15 – 10.45 am ***Coffee Break***

Symposium V: Functional Genomics

10.45 – 11.00 am ***Genome sequencing of childhood medulloblastoma brain tumors links chromothripsis with TP53 mutations***
Jan Korbel

Jan Korbel is a group leader in the Genome Biology Research Unit of the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. Jan pursued his Ph.D. research at EMBL Heidelberg, and his postdoctoral research at Yale University in New Haven, CT, USA. Over the past few years the Korbel group has been developing massively-parallel sequencing technology and computational biology based approaches to characterize the mechanisms of formation and functional consequences of genetic variation, particularly genomic structural variation, in the normal population and in the context of human diseases (specifically cancer). One main research focus is dissecting the molecular basis of complex forms of structural variation using computational biology and molecular biology. A particular interest of the group lies in understanding the mechanistic basis of a cellular process in which massive SVs form in a single catastrophic event, termed chromothripsis – a crucial event during the development of childhood medulloblastomas of the Sonic Hedgehog-driven subtype.

11.00 – 11.15 am ***Secretion of Wnts is required for Wnt/ß-catenin pathway activity in colorectal cancer (CRC) despite APC or ß-catenin mutations***
Gerrit Erdmann

Gerrit Erdmann started studying molecular biotechnology at Heidelberg University in 2003 and received his Bachelors degree in 2006. He then continued his studies in Heidelberg receiving his Masters degree in molecular and cellular biology in 2008. During his Master thesis he worked on tamoxifen resistance in breast cancer. In 2008, Gerrit received an HBIGS PhD fellowship and joined the Lab of Prof. Michael Boutros at the DKFZ. There, he worked on the Wnt/β-catenin signaling pathway in colon cancer and mesenchymal stem cells. During his PhD he participated in the NGFN consortium “Integrated Genomic Investigation of Colorectal Carcinoma” utilizing high-throughput screening and next generation

sequencing. Gerrit finished his PhD in September 2012 and is continuing his work in the lab of Prof. Boutros as a post-doc. His research interests are focused on understanding signaling pathways and their aberrant regulation in cancer as well as their role in stemness and differentiation.

11.15 – 11.30 am ***LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression*.**
Sven Lindner

Sven Lindner is a Ph.D. student in the Laboratory for Pediatric Oncology Research II (Head: Prof. Dr. J.H. Schulte) in the department of Pediatric Hematology and Oncology (Director: Prof. Dr. A. Eggert) at the University Hospital Essen, Germany. In his master thesis in biology, done at the Ruhr University Bochum, Germany, he studied the role of neural crest cells in neuroblastoma development.
His research interest is the molecular biology of neuroblastoma, the most common extracranial tumor of childhood. He is analyzing the role of oncogenes in neuroblastoma and their influence on neural crest cells in his doctoral research. To date, his research has contributed to identifying FOXR1 (Oncogene 2011) and LIN28b (Nat Genet 2012) as oncogenes in neuroblastoma and the creation of a LIN28b mouse model, the establishment of a cellular model for validating potential neuroblastoma oncogenes in neural crest cells, and the validation of an ALK mutant using this model (SciTransl Med 2012, Oncogene 2012).

11.30 – 11.45 am ***Human BCL2-associated athanogene 3 (BAG3) mutations lead to dilated cardiomyopathy in zebrafish***
Sören Westphal

Sören Westphal, M.D., studied medicine from 1997 to 2004 at the Medical University of Lübeck and passed his Doctoral Thesis in 2006. During his studies he was recipient of a scholarship of the German National Academic Foundation. Besides his residency training in the field of internal medicine his initial scientific work was focused on the pathophysiology of the Metabolic Syndrome. During this period he received the Best Poster Award at the 37th annual meeting of the German Diabetes Society. Since 2011 Sören Westphal is postdoctoral research fellow in the Molecular Cardiology Group of Prof. Wolfgang Rottbauer at the University Hospital of Ulm. He devoted his research to elucidate novel aspects of developmental heart biology and the pathogenesis of cardiomyopathies. In particular he conducts functional genomics to identify the biological significance of genome-wide association studies (GWAS) findings in vivo using the zebrafish model organism. Besides of these studies he is interested in regenerative medicine and novel pharmacological therapeutic approaches. Sören Westphal is member of the German Cardiac Society.

11.45 – 12.00 pm ***Cyp17a1 deficient mice display increased body weight, visceral/subcutaneous fat deposition and altered lipid metabolism***
Zouhair Aherrahrou

Zouhair Aherrahrou is currently working as group leader in the newly founded Institut für Integrative und Experimentelle Genomik at the Universität zu Lübeck. Mainly his research focuses on cardiovascular diseases and their main complication, myocardial infarction (MI). The special interest of his research agenda lies on revealing genetic determinants that control this common complex disorder in mouse models and human, and functionally determine the role of these genes in respective disorders. Especially Zouhair Aherrahrou initiated the

CardioGENE Knockout pipeline project to generate and phenotype transgenic mouse models for the recently identified coronary artery disease risk genes.
He studied biology in Morocco at the University Moulay Esmail, Méknès and University Sidi Med Ben Abdellah, Fés (1986-1991) and accomplished his Master Degree in Pharmacy at China Pharmaceutical University, Nanjing, China (1994-1997). He accomplished his PhD thesis in human biology at the Universität zu Lübeck, Germany (2001-2004). In 2002, he finished his training in mouse genetics at Pasteur Institute, Paris, France.
His research has been funded by NGFN2 and NGFNplus.

12.00 – 02.00 pm ***Lunch Break and Poster Session II***

Company Satellite Sessions *(Conference Room K1)*

12.10 – 12.40 am **Raimo Tanzi, Life Technologies GmbH**
Ion torrent next Generation sequencing technology: the revolution of the revolution

12.45 – 01.15 pm **Janine Altmüller, Roche Diagnostics Deutschland GmbH**
Whole exome sequencing (WES) speeds up gene identification in monogenetic human diseases

01.20 – 01.50 pm **Rebekka Krumbach, SEQUENOM GmbH**
Translating Genomic Discovery into Human Health - The MassARRAY® for Somatic Mutation Profiling in Cancer and Quality Control for NGS Projects

01.55 – 02.25 pm **Jim White, Nanostring Technologies**
Gene Expression Analysis Down to the single Cell level by Digital Quantification of Nucleic Acids Utilizing a Color-Coded Barcode Technology

Poster Award Ceremony

02.00 – 02.15 pm **Ceremony: "Annemarie Poustka Poster Award 2012"** sponsored by Roche Diagnostics GmbH
Christine Kuch, Roche Diagnostics Deutschland GmbH
Hugo A. Katus, Heidelberg University Clinics, Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

Symposium VI: Personalized Medicine

02.15 – 02.45 pm **Keynote Presentation:**
Systems approaches to Parkinson´s disease
Rudi Balling

Prof Balling is a geneticist. He studied nutrition at the Universities of Bonn and Washington State University, USA and received his PhD in Human Nutrition from the University of Bonn, Germany. In 1993, he became Director of the 'Institute of Mammalian Genetics' at the GSF National Research Center for Environment and Health in Munich, after completing research posts at Mount Sinai Research Hospital in Toronto, Canada and the Max Planck Institutes of Biophysical Chemistry in Göttingen and Immunbiology in Freiburg, Germany. In 2001, he took over the position as Scientific Director of the Helmholtz Centre for Infection Research in Braunschweig, a research center with close to 700 employees. Prior to his appointment as Director or the LCSB, Professor Balling was a guest professor at the Broad Institute of MIT/Harvard University in Boston (USA).

02.45 – 03.00 pm ***A promising therapy strategy for PDAC: MEK1/2 inhibition with the novel chemotherapeutic drug BAY 86-9766 (RDEA119)***
Nicole Teichmann

Nicole Teichmann started her academic studies in biology at Friedrich Schiller University Jena in 2005 and received her diploma in 2010. Within the scope of her Diploma thesis she investigated the cell cycle regulation mediated by the cancer biomarker candidate S100A11. In 2010, she joined the group of PD Dr. Jens Siveke at Klinikum rechts der Isar der Technischen Universität München as a PhD student of the International Max Planck Research School for molecular and cellular life sciences (IMPRS-LS). There, her work is focussed on preclinical evaluation of novel chemotherapeutic agents for pancreatic cancer using endogenous mouse models. Moreover, she is interested in understanding the aberrant regulation of signaling pathways in pancreatic cancer as well as their role in mediating resistance to therapies. During her PhD Nicole Teichmann presented her data at several national and international conferences and received the National Scholar Award of UEG Week 2012 in Amsterdam.

03.00 – 03.15 pm ***Haplotype-resolving multiple human genomes: Key to personalized medicine and genome biology***
Margret Hoehe

Dr. Margret Hoehe, Genome Research Group Leader, MPI Molecular Genetics, Berlin, has been a pioneer in research on human genetic individuality. She has demonstrated inter-individual variation in drug response and evidence for genetic vulnerability markers at the University of Munich in the 80's; identified the first polymorphisms of receptor genes and linkage to psychiatric disorders at the NIMH/NIH; developed since 1992 with George Church at Harvard a Harvard "Multiplex PCR Sequencing" technology to perform large scale analysis of DNA sequence differences, and was in 2000 one of the first two to demonstrate importance of haplotypes over SNPs. She & her team have developed fosmid pool-based key technologies and resources to haplotype-resolve whole genomes, and reported in 2011 the two most completely haplotype-resolved human genomes to date. Her current focus is the analysis of haplotype-resolved genomes at the population level to advance diploid genomics as the foundation for human biology and individualized medicine, and "phase-sensitive" personal genomics.

03.15 – 03.30 pm ***The risk of myoacardial infarction is increased by digenic mutation in GUCY1A3 and CCT7 - identified by exome sequencing in an extended family***
Jeanette Erdmann

Jeanette Erdmann, born 1965, is since October 2012 W3-Professor for „kardiovaskuläre Molekulargenetik“ at the Universität zu Lübeck. Starting in January 2013 she will be head of the newly founded „Institut für Integrative und Experimentelle Genomik“ in Lübeck.
Since 1991 she is interested in unravelling the genetics of complex diseases, starting as a PhD in the field of psychiatric disorders at the Institut für Humangenetik in Bonn under the supervision of Prof. Propping and Prof. Nöthen. As a PostDoc (in Berlin, Regensburg) she started to work in the field of cardiovascular diseases. In 2004 she moved to Lübeck and together with Prof. Schunkert she established a very sucessful working group for „Cardiovascular Molecular Genetics“ at the Universität zu Lübeck. She has published over 120 manuscripts (IF=1.400) and is involved as a principle investigator in the large-scale meta-analysis consortia for coronary artery disease (CAD) and myocardial infarction (MI) (CARDIoGRAM and CARDIoGRAMplusC4D). Together with colleagues from these consortia she was involved in the identification of almost all known genetic risk loci for CAD and MI.

03.30 – 03.45 pm ***High-throughput cell-based assays identify Placenta-specific 8 (Plac8; Onzin) as a key regulator of proliferation and survival in pancreatic cancer cells***
Brajesh Kaistha

Dr. Brajesh Pratap Kaistha was born in the sub-Himalayan town of Dharamshala in India. After completing his Bachelors in Biology and Masters in Microbiology/Biochemistry from HPKV University in Himachal Pradesh, India, he came to Germany to pursue higher education. He obtained his PhD in Biology from Philipps University Marburg in year 2010 and is currently working as postdoctoral scientist in Dept. of Medicine in Philipps University Marburg. He is currently involved in studying the biology of pancreatic ductal adenocarcinoma (PDAC) to get a deeper mechanistic insight. Using a variety of cutting edge molecular biology and biochemical techniques together with transgenic mice approaches, he together with his colleagues is focussed on identifying and characterising novel genes involved in the pathogenesis of PDAC and thereby identifying new options for therapeutic interventions.

03.45 – 04.00 pm **Concluding Remarks: Markus Nöthen**, Friedrich-Wilhelms University, Bonn, Germany, Spokesperson Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

04.00 – 04.30 pm **Closing Coffee**

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Overviews

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93	I-Keynote-3	Matthew Meyerson	Genome Alterations in Human Lung Cancers	ICGC
94	I-Keynote-4	Hans Lehrach	1000 Genomes Project – An integrated map of genetic variation	1000 Genomes Project
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99	II-Keynote-1	John Blangero	Identification of Complex Disease Genes Using Whole Genome Sequencing in Large Pedigrees	
100	II-Keynote-2	Joris A. Veltman	De novo diagnostics in patients with intellectual disability	
101	II-Keynote-3	Gerard D. Schellenberg	Neurodegenerative disease genetics; GWAS, exomes and beyond	ADGC
102	II-Keynote-4	Peter Jones	The Cancer Epigenome	IHEC
103	II-Keynote-5	Jörn Walter	Control of DNA-methylation in development	IHEC
104	II-Keynote-6	Wolfgang Wurst	International Mouse Knock-out Consortium: Resource to functionally validate all genes	EUCOMM
105	II-Keynote-7	Martin Hrabě de Angelis	Creating a comprehensive encyclopedia of mammalian gene function	EUMODIC
106	II-Keynote-8	Matthias Mann	High resolution, quantitative mass spectrometry combines proteomics and genomics	
Symposium III - Genomics of Common Disease I				
111	O-III-1	André Reis	Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study	Mental retardation
112	O-III-2	Vanessa Nieratschker	Pre-, peri- and postnatal Stress in human and non-human off-spring: a convergent approach to study genetic and epigenetic impact on depression	Schizophrenia
113	O-III-3	Andre Franke	Host-microbe interactions shape genetic risk for inflammatory bowel disease	Environment-related diseases
114	O-III-4	Robert Häsler	Mapping the Functional Epigenome of Ulcerative Colitis in Monozygotic Twins	Environment-related diseases
115	O-III-5	Anke Hinney	Childhood ADHD and obesity: evidence for a common genetic link	Obesity
116	O-III-6	Ralf Herwig	An integrated catalogue of genome, methylome and gene expression variations in colon cancer	Colon cancer
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123	O-IV-2	Martin Peifer	Toward a novel genomics-based taxonomy of lung cancer: The Clinical Lung Cancer Genome Project	Cancer genes
124	O-IV-3	Christel Herold-Mende	Aberrant Self-Renewal and Quiescence Contribute to Glioblastoma Aggressiveness	Brain tumor network
Symposium V - Functional Genomics				
129	O-V-1	Jan Korbel	Genome sequencing of childhood medulloblastoma brain tumors links chromothripsis with TP53 mutations	ICGC PedBrain Tumor
130	O-V-2	Gerrit Erdmann	Secretion of Wnts is required for Wnt/ß-catenin pathway activity in colorectal cancer (CRC) despite APC or ß-catenin mutations	Colorectal cancer
131	O-V-3	Sven Lindner	LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression.	Neuroblastoma
132	O-V-4	Sören Westphal	Human BCL2-associated athanogene 3 (BAG3) mutations lead to dilated cardiomyopathy in zebrafish	NGFN-2 / -1
133	O-V-5	Zouhair Aherrahrou	Cyp17a1 deficient mice display increased body weight, visceral/subcutaneous fat deposition and altered lipid metabolism	Atherogenomics
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138	O-V-1	Nicole Teichmann	A promising therapy strategy for PDAC: MEK1/2 inhibition with the novel chemotherapeutic drug BAY 86-9766 (RDEA119)	Pancreatic cancer
139	O-V-2	Margret R. Hoehe	Haplotype-Resolving Multiple Human Genomes: Key to Personalized Medicine and Genome Biology	MHC Sequencing
140	O-V-3	Jeanette Erdmann	The Risk of Myoacardial Infarction is increased by Digenic Mutation in GUCY1A3 and CCT7 - identified by exome sequencing in an extended family.	Atherogenomics
141	O-V-4	Brajesh Kaistha	High-throughput cell-based assays identify Placenta-specific 8 (Plac8; Onzin) as a key regulator of proliferation and survival in pancreatic cancer cells	Pancreatic cancer

National Genome Research Network

List of Poster Abstracts

sorted by symposia

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

Poster Session I:

Wednesday, December 12th, 2012

Odd numbers: 12.00 - 01.00 pm
Even number: 01.00 - 02.00 pm

Poster Session II:

Thursday, December 13th, 2012

Odd numbers: 12.00 - 01.00 pm
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148	P-III-2	Josef Frank	Association of NPY Receptor 2 Polymorphism with Alcohol Dependence	Alcohol addiction
149	P-III-3	Ildikó Rácz	Long-term ethanol effects on acute stress responses: Modulation by dynorphin	Alcohol addiction
150	P-III-4	Anne Richter	Genetic variation in the artrial natriuretic peptide transcription factor GATA4 modulates amygdala responsiveness to alcohol cues und relapse risk in alcohol-dependent patients	Alcohol addiction
151	P-III-5	Jens Treutlein	Convergent Functional Genomics in Alcohol Addiction Research - A Translational Approach to Identify and Study New Candidate Genes	Alcohol addiction
152	P-III-6	Wei Gu	Genome wide association study of recently found new Alzheimer candidate genes in a German cohort combined with endophenotypes	Alzheimer's disease
153	P-III-7	Sven Reinhardt	Computational identification and experimental validation of microRNAs binding to the Alzheimer-related gene ADAM10	Alzheimer's disease
154	P-III-8	Matthias Riemenschneider	Several promoter CpGs are strongly associated with beta amyloid levels in post-mortem Alzheimer`s disease brain tissue	Alzheimer's disease
155	P-III-9	Matthias Riemenschneider	Genome-wide microRNA expression profiling in Alzheimer’s disease	Alzheimer's disease
156	P-III-10	Corinna Roth	Generating a Reporter mouse which allows facilitated assessment of ADAM10 transcriptional activity in vivo	Alzheimer's disease
157	P-III-11	Felicitas. Becker	The clinical spectrum of PRRT2 mutations - new mutations in PKD, ICCA and BFIS	Epilepsy and Migraine
158	P-III-12	Tobias Freilinger	Migraine without aura: genome-wide association analysis identifies several novel susceptibility loci	Epilepsy and Migraine
159	P-III-13	Tanja Grimminger	Genomic analyses of Levetiracetam resistance in human epileptic tissue	Epilepsy and Migraine
160	P-III-14	Katharina Pernhorst	Rs6295 promoter variants of the serotonin type 1A receptor correlate to respective mRNA expression in human epileptic brain tissue	Epilepsy and Migraine
161	P-III-15	Mariana Zaichuk	Functional analysis of novel SCN2A mutations found in patients with infantile epilepsies.	Epilepsy and Migraine
162	P-III-16	Martin H. Schaefer	Integrating protein-protein-interaction networks with experiment-based quality scores and context information for the detection of reliable and meaningful interactions	Neurodegenerative diseases
163	P-III-17	Claudia Schulte	Novel Variants in autosomal dominant Parkinson’s disease: Exome Sequencing and Linkage Analysis	Neurodegenerative diseases
164	P-III-18	Erich Wanker	The UBX protein ASPL rapidly converts functional p97 hexamers into non-functional heterotetramers	Neurodegenerative diseases
165	P-III-19	Meike Diepenbroek	Impact of calpain cleavage of alpha-synuclein on the pathogenesis of Parkinson's disease in vivo	Parkinson's disease
166	P-III-20	Florian Giesert	Gain- and Loss-of LRRK2 – Comprehensive analysis of two Mouse Models	NGFN-2 / -1

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168	P-III-22	Franziska Degenhardt	Duplications in RB1CC1 are associated with schizophrenia in large samples from Europe	Schizophrenia
169	P-III-23	Andreas J. Forstner	Clustering Analysis of Low-Frequency Risk Variants in the Schizophrenia-associated 1q21.1 Microdeletion Region Suggests CHD1L as Disease-Relevant Gene	Schizophrenia
170	P-III-24	Barbara Heinemann	Analysis of copy number variants in genes reported to carry a genome-wide significant SNP	Schizophrenia
171	P-III-25	Dilafruz Juraeva	Pathway analysis of two GWAS cohorts for unipolar disorder	Schizophrenia
172	P-III-26	Sandra Meier	Studies in humans and mice implicate neurocan in the etiology of mania	Schizophrenia
173	P-III-27	Thomas W. Mühleisen	Genome-wide association study reveals four new risk loci for bipolar disorder	Schizophrenia
174	P-III-28	Jana Strohmaier	Alopecia Areata: Genetic and Psychological Factors	Schizophrenia
175	P-III-29	Stephanie H Witt	Transcription profiling and pathway analysis in euthymic and manic bipolar patients and controls	Schizophrenia
176	P-III-30	Ingrid Brænne	Whole-exome sequencing in extended families with myocardial infarction	Atherogenomics
177	P-III-31	Christina Willenborg	Coronary artery disease risk loci identified in over 190,000 individuals implicate lipid metabolism and inflammation as key causal pathways	Atherogenomics
178	P-III-32	Katja Grabowski	Gene expression program during cardiac growth in SHRSP shows variability associated with hypertensive left ventricular hypertrophy	Heart Failure
179	P-III-33	Frank Rühle	Advanced GWAS interpretation in R	Heart Failure
180	P-III-34	Britta Vogel	Dissecting the Role of microRNAs as Biomarkers for Cardiovascular Diseases	Heart Failure
181	P-III-35	Michaela Müller	Homology model of Abcc6 provides insight into the function of mutations causing cardiovascular phenotype	NGFN-2 / -1
182	P-III-36	Nadja Knoll	Common variation in mitochondrial DNA is not associated with obesity	Obesity
183	P-III-37	Nadine Rink	Metabolic Phenotyping of the Mouse Mutant Line Ftotm1b	Obesity
184	P-III-38	André Scherag	1000G imputations in a family-based genome-wide association study for genomic imprinting of early onset extreme obesity	Obesity
185	P-III-39	Mandy Stadion	A microdeletion on chromosome 1 of C57BL/6J mice results in a loss of function of Ifi202b which suppresses 11ß-hydroxysteroid dehydrogenase type 1 expression and development of obesity	Obesity
186	P-III-40	Anna-Lena Volckmar	Mutation screen in the GWAS derived obesity gene SH2B1 including functional analyses of detected variants	Obesity
187	P-III-41	Thomas W Winkler	Genome-wide meta analysis across 270,000 individuals identifies seven sexually dimorphic variants associated with human anthropometric traits	Obesity

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189	P-III-43	Hansjörg Baurecht	Meta-Analysis of Genome-wide Association Studies on Atopic Dermatitis Identifies Three Novel Risk Loci	Environment-related diseases
190	P-III-44	Nadezhda T. Doncheva	Prioritization and Functional Characterization of Genes in Disease-Specific Networks	Environment-related diseases
191	P-III-45	Eva Ellinghaus	Dense genotyping of known immune-mediated disease regions identifies nine new risk loci for primary sclerosing cholangitis	Environment-related diseases
192	P-III-46	Georg Hemmrich-Stanisak	Towards blood-born microRNA biomarkers for inflammatory bowel disease	Environment-related diseases
193	P-III-47	Simone Lipinski	RNAi screening identifies FRMPD2: a scaffolding protein involved in basolateral NOD2 signaling	Environment-related diseases
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National Genome Research Network

Oral Presentation Abstracts

National Genome Research Network

Oral Presentation Abstracts

Symposium I

International Projects in the Program of Medical Genome Research

ICGC

Translation of the Cancer Genome

Presenting Author: Lynda Chin

University of Texas, MD Anderson Cancer Center, Houston, TX

With advances in international and national efforts to characterize cancer genomes, the focus of cancer genomics is evolving from generation of comprehensive epi/genomic and proteomic data as a snapshot reference to practical application in the clinics and impact in the practice of medicine. The integration of longitudinal genomic profiling of both germline and somatic genomes with patient history and clinical information, enabled by health IT technology innovation, will provide new opportunities to transform cancer research and impact the care of patients. However, successful translation of these insights into drugs and tests that can impact on patient outcomes will still require deep biological understanding of the complexity and adaptive nature of cancer. Examples of some of these efforts will be discussed.

ICGC

Novel findings in pediatric brain tumors revealed by high resolution genome, methylome and transcriptome analyses

Presenting Author: Peter Lichter on behalf of the PedBrain Tumor Network

Division of Molecular Genetics, German Cancer Research Center, Heidelberg, Germany

Brain tumors are the second most common pediatric cancer and carry the highest mortality rates in this age group. Pilocytic astrocytoma is the most frequent benign and medulloblastoma the most frequent malignant brain tumor of childhood. Glioblastoma, a brain tumor that carries a dismal prognosis, displays considerable heterogeneity. Within the ICGC project „PedBrain“ we have carried out comprehensive genome, methylome and transcriptome analyses in large cohorts of these three pediatric tumor entites applying next-generation DNA sequencing. These studies revealed a wealth of new findings including i) refinement of tumor classification schemes, ii) elucidation of novel pathomechanistic aspects of genome instability, and iii) identification of novel pathways and actionable targets. Recent findings related to current challenges in translational oncology will be presented.

ICGC

Genome Alterations in Human Lung Cancers

Presenting Author: Matthew Meyerson

Dana-Farber Cancer Institute; Department of Pathology, Harvard Medical School; and Broad Institute of Harvard and MIT

Cancer is a disease of the genome. High-throughput genome analysis tools now enable the detection of somatic alterations in cancer cells including point mutations, copy number alterations, translocations, and infections. Our approaches include next-generation sequencing of cancer genomes, exomes, and transcriptomes as well as single nucleotide polymorphisms (SNP) array analysis of copy number. We are also participating in "The Cancer Genome Atlas" or TCGA-project of the National Institutes of Health, which aims to characterize the genomes of 10,000 human cancers.
In this presentation, I will discuss analysis of the genomes of human lung cancers, with a focus on data from lung adenocarcinomas and squamous cell lung carcinomas. I will describe recently published results on integrated profiling of squamous cell lung carcinoma genomes from TCGA and exome and genome sequence analysis of lung adenocarcinoma, as well as an update of the comprehensive TCGA analysis of lung adenocarcinomas.

1000 Genomes Project
An integrated map of genetic variation

Presenting Author: Hans Lehrach

MPI for Molecular Genetics, Berlin, Germany

Since August 2008, the Max Planck Institute for Molecular Genetics (MPIMG) in Berlin has been participating in the international 1000 Genomes project. The international Human Genome Project has determined the reference sequence of the human genome. With the advent of next generation sequencing technologies, it has become possible 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 phenotypes are generated. In response to this, the 1000 Genomes Project was launched in 2008. The aim of the project is to discover genotype and provide accurate haplotype information on all forms of human DNA polymorphism in multiple human populations. During the project pilot phase, three studies were conducted to test multiple strategies for producing a catalogue of genetic variants that are present in 1 percent or more of the different populations chosen for study (European, African and East Asian). The subsequent production phase of the full project combines low coverage whole genome sequencing, array based genotyping and deep targeted sequencing of all coding regions in 2,500 individuals from 26 populations of the world. This project will be key to a further understanding of genetic variation and will help geneticists and physicians to learn more about the role of individual variations in the development of diseases.

National Genome Research Network

Oral Presentation Abstracts

Symposium II

International Projects in Genome Research

Identification of Complex Disease Genes Using Whole Genome Sequencing in Large Pedigrees

Presenting Author: John Blangero

Texas Biomedical Research Institute, San Antonio, TX, USA

Data are rapidly accumulating that rare variants likely have a large cumulative effect on normal phenotypic variation and are important in disease risk. Whole genome sequencing (WGS) in large extended pedigrees represents the optimal design for identifying rare causal variants because Mendelian transmission from parents to offspring increases the chance that multiple copies of rare variants will be observed in a pedigree. A pedigree-specific rare functional variant with small relative effect size in relation to population attributable risk or locus-specific heritability, but with a larger absolute effect size, can be sufficient to verify that a given gene is causally involved in relevant phenotypic variation. In this talk, I will describe how WGS can be used in large pedigrees to rapidly identify genes causally involved in human disease-related variation. The analysis of such data in pedigrees requires special methods that can take into account the non-independence amongst family members due to shared genetic kinship. Using some of the world's very first WGS data from large Mexican American pedigrees, I'll provide examples of the utility of both the sampling design and analytical approaches for gene discovery.

In the T2D-GENES Project, we now have WGS on approximately 1000 Mexican American individuals who have been studied for type 2 diabetes and related phenotypes. The amount of rare (and even private) genetic variation seen in these pedigrees is striking. We have currently observed approximately 25M sequence variants. Because of the power-leaching effects of testing so many potential variants, we employ strategies for identifying high quality prior hypotheses for reducing the causal search space. For example, restricting our focus to protein-altering coding variation, we observed an average of ~9600 non-synonymous variants per individuals of which 15% are predicted to be potentially damaging to the focal protein. We identified approximately 28,000 private loss of function and coding variants. These numbers suggest the substantial likelihood that rare coding variants play a significant role in the phenotypic variation observed in any given biological pathway. Similarly, likely rare regulatory variation identified by both bioinformatic and empirical transcriptional profile data are also found in substantial numbers and can serve as a useful hypothesis-driven filter for testing. Examples of human quantitative trait loci (QTLs) being identified using this approach in the remarkable data set will be provided.

De novo diagnostics in patients with intellectual disability

Presenting Author: Joris A. Veltman

Department of Human Genetics, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Germline coding de novo mutations (SNVs, indels as well as CNVs) are an important cause of moderate to severe forms of intellectual disability (ID) and associated syndromes. Exome sequencing now allows us to reliably identify these mutations using a single genomic test. In this presentation, I will first discuss the role of de novo mutations in genetic disease and the associated risk factors such as the local genomic structure and paternal age. Next I will describe our recent work on using a family-based exome sequencing approach to test this de novo mutation hypothesis in 100 patients with unexplained ID, as well as targeted follow-up studies of several candidate ID genes in 750 additional patients. A total of 79 unique coding de novo mutations were identified and validated in 52 patients. Damaging de novo (n=10) as well as X-linked maternally-inherited (n=3) mutations were detected in known ID genes, resulting in the detection of 13 pathogenic mutations (13%) in this cohort. In addition, potentially causative de novo mutations in novel candidate ID genes were detected in 22 patients. For three of these candidate genes, recurrent de novo mutations were identified in patients with similar phenotypes, confirming that they are true ID genes. To further expand the possibilities of diagnostic exome sequencing for mutation detection we have recently validated automatic CNV detection on exome data, and compared its performance to that of high resolution genomic microarrays. This analysis shows that exome sequencing can reliably detect the large majority of pathogenic de novo CNVs, responsible for and additional ~15% of ID. In conclusion, de novo mutations represent an important cause of ID and exome sequencing is an effective diagnostic strategy for their detection. Related to this, exome sequencing is also of great diagnostic value for other genetically heterogeneous disorders like blindness, deafness, movement disorders, mitochondrial disorders and unexplained genetic syndromes.

ADGC - Neurodegenerative disease genetics; GWAS, exomes and beyond

Presenting Author: Gerard D. Schellenberg

University of Pennsylvania School of Medicine, Philadelphia, USA

Genome-wide genetic approaches are now being successfully used to understand the genetic architecture of Alzheimer's disease (AD). Early work identified APOE as a major susceptibility gene for late-onset AD (LOAD). Genetic analysis of the APOE show that the signal from this gene comes entirely from the ε2/ε3/ε4 polymorphism and no other variants in the region. Genome-wide association studies (GWAS) performed by the Alzheimer's disease Genetics Consortium (ADGC) and others have now identified 9 different loci that contribute to AD susceptibility. These are ABCA7, BIN1, CD2AP, CD33, CR1, CLU, EPHA1, MS4A4/MS4A6E, and PICALM. Recently, using combined Caucasian and Japanese cohorts, we show that SORL1, a gene previously implicated by candidate gene studies, is significant at the genome-wide level. We have also extended these findings in to an African America population. To increase power to detect AD loci, we formed the International Alzheimer Genomic Project (IGAP) which is an international collaboration of all major AD genetics groups. We performed a mega-meta analysis using 17,008 AD cases and 45,962 controls. We identified 4 new AD genes. In a replication study, we designed a custom genotyping array of SNPs that had a $P < 10\text{-}3$ and are genotyped an additional 14,000 cases and 14,000 controls. The next phase of analysis is to identify rare variants that contribute to AD risk using using exome chips, whole exome sequencing, and whole genome sequencing. Whole-genome approaches for AD and other neurodegenerative disorders (e.g. progressive supranuclar palsy) will hopefully lead to the identification of new AD therapeutic targets.

The Cancer Epigenome

Presenting Author: Peter Jones

Department of Urology and Biochemistry & Molecular Biology, USC Norris Comprehensive Cancer Center, Keck School of Medicine of USC, Los Angeles, CA

Recent genome sequencing of a large number of human tumors has shown an unexpectedly high frequency of mutations in genes which establish and interpret the epigenome. The mutations occur in epigenetic writers such as EZH2 and DNMT3A, readers such as BRD4-NUT fusions and in erasers (UTX), and also chromatin remodelers such as the BAF subunits of SWI/SNF. Because the mutations occur at high frequencies they may be drivers of carcinogenesis and this has been shown functionally in some cases. This data has brought the role of epigenetics in human cancer to the fore and suggests a close collaboration between genetic and epigenetic events in carcinogenesis (You and Jones, 2012). The realization that epigenetic pathways are fundamentally altered in neoplasia has spurred the development of drugs which will target these defects and likely will be therapeutic for specific types of cancers. There is therefore a need to map the genetic and epigenetic landscapes in cancer to provide a backdrop for the rapid development and clinical utilization of new epigenetically targeted therapies.

Projects such as the International Human Epigenome Consortium have shown that it is now possible to map human epigenomes in great detail and several projects are currently underway to achieve this task in normal tissues. Mapping of cancer epigenomes is more complicated but one which is clearly attainable using currently available technologies and likely to become increasingly achievable over the next few years. The American Association for Cancer Research (AACR) has established a cancer epigenome task force which recommends the development of a private-public partnership (The International Cancer Epigenome Project, ICEP) to map a defined number of cancer epigenomes which may be relevant to the development of new therapeutics. Mapping of cancer epigenomes on a large scale will also show the inter- and intra-case variability between and within tumors and have important implications for our understanding of the epidemiology, detection, prevention and treatment of cancer.

You, J.S., and Jones, P.A. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 22:9-20, 2012.

IHEC - Control of DNA-methylation in development

Presenting Author: Jörn Walter

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Methylation of DNA-bases is a key epigenetic signature that is linked to the dynamic control of chromatin structure and function in the human genome. DNA-methylation is one of the core layers of epigenetic information currently investigated in various epigenome programs for primary human cells. The goal of these projects gathered under the umbrella of the international human epigenome consortium IHEC is to provide a comprehensive reference catalogue of cell specific epigenomic maps using high resolution NGS based technologies. The complex data obtained challenge the „traditional" concepts of epigenetic control. Particularly the recent detection of novel oxidative forms of DNA modifications reveal a new perspective on the heritable role and functional control by DNA-methylation. In our group we use ultradeep sequencing methods to uncover the dynamics of DNA-methylation at critical developmental stages and in diseases models. In my presentation I will our recent finding on mechanisms leading to the resetting and inheritance, respectively, of DNA-methylation in early developmental phases and in stem cells.

Arand J, Spieler D, Karius T, Branco MR, Meilinger D, Meissner A, Jenuwein T, Xu G, Leonhardt H, Wolf V, Walter J. In vivo control of CpG and non-CpG DNA methylation by DNA methyltransferases. PLoS Genet. 2012 Jun;8(6):e1002750.

Wossidlo M, Nakamura T, Lepikhov K, Marques CJ, Zakhartchenko V, Boiani M,Arand J, Nakano T, Reik W, Walter J. 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. Nat Commun. 2011;2:241. Wossidlo M, Arand J, Sebastiano V, Lepikhov K, Boiani M, Reinhardt R, Schöler H, Walter J. Dynamic link of DNA demethylation, DNA strand breaks and repair in mouse zygotes. EMBO J. 2010 Jun 2;29(11):1877-88.

Lepikhov K, Wossidlo M, Arand J, Walter J. DNA methylation reprogramming and DNA repair in the mouse zygote. Int J Dev Biol. 2010;54(11-12):1565-74.

EUCOMM - International Mouse Knock-out Consortium: Resource to functionally validate all genes

Presenting Author: Wolfgang Wurst

Helmholtz Zentrum Munich, Germany

The decoding of the sequences of the human and mouse genomes has been heralded as an historical milestone. This massive accomplishment has required the concerted action of the international scientific community, who have joined forces, exchanged technical and scientific knowledge and pooled resources. Analysis of the mouse and human genomes has resulted in the identification of approximately 21.000 coding genes as well as of thousands of conserved non-coding regions. Now attention has turned to the next phase of the project, elucidation of gene function in the context of the entire organism in a coordinated large scale fashion.

As part of an international knockout Mouse Consortium (IKMC http://www.knockoutmouse.org/), the European Conditional Mouse Mutagenesis Program EUCOMM/EUCOMMTOOLS produces conditional mutations throughout the mouse genome in a systematic high throughput way. In total, a collection of more than 17.000 conditionally mutated coding genes have been generated in mouse embryonic stem (ES) cells using conditional gene targeting and trapping approaches. In addition, about 400 miRNA loci have been mutated as well. From these resources, more than 1.900 mouse mutants have been generated for phenotypic analysis which is recently performed in the framework of the International Mouse Phenotyping Consortium (IMPC). Furthermore, we are currently establishing 250 Cre-driver lines to take full advantage of the conditional EUCOMM alleles to determine gene function in a given cell of the body at any time. This IKMC library will enable mouse mutants to be established worldwide in a standardized and cost-effective manner, making mouse mutants available to a much wider biomedical research community than has been possible previously. All material including targeting vectors, mutant ES cells, mouse resources, and Standard Operating Procedures (SOPs) are being displayed to the scientific community via the International Knock-out Mouse Consortium web site (www.knockoutmouse.org). Vectors and ES cells are distributed by the European Mouse Mutant Cell Repository (www.EUMMCR.org), and mice by the European Mouse Mutant Archive (EMMA, www.emmanet.org).

To enhance gene targeting technology, we further developed zinc-finger nucleases and TALENs for the application in one-cell mouse embryos to generate disease-related mutants harboring single nucleotide or codon 1replacements directly in the germ line. First results demonstrate the feasibility of seamless gene editing in one-cell embryos to create genetic disease models as a simplified mutagenesis tool.

Thus, these genetic tools will greatly facilitate gene function annotation and disease modeling.

EUMODIC and INFRAFRONTIER - Creating a comprehensive encyclopedia of mammalian gene function

Presenting Author: Martin Hrabě de Angelis and the German Mouse Clinic Consortium

German Mouse Clinic, Helmholtz Zentrum Munich, Germany

One of the most important tools at our scientific disposal in understanding mammalian gene function is the laboratory mouse. The scientific community has taken advantage of its fundamental similarity to humans at the genetic level (>95% at the gene level), similar physiology and anatomy, its relative low cost compared to other mammals, and nearly 100 years of genetic study.
The International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org) was recently created to carry out high-throughput systemic phenotyping of knockout mice for every gene in the mouse genome (20,000 plus genes) in order to create a comprehensive encyclopedia of mammalian gene function. The IMPC builds on the efforts of International Knockout Mouse Consortium (IKMC, www.knockoutmouse.org) to systematically generate mutant ES cells for every gene in the mouse genome. All data will be freely available to the scientific community.
The German Mouse Clinic (GMC, www.mouseclinic.de) was founding member and contributes to the IMPC in the context of INFRAFRONTIER, the European Research Infrastructure for Phenotyping and Archiving of Model Mammalian Genomes (www.infrafrontier.eu). The GMC pioneered the systemic analysis of mouse models and was already involved in the pilot program to the IMPC, the European EUMODIC consortium, which showed the feasibility of generating and phenotyping 500 mouse strains in a high-throughput fashion. As before in EUMODIC, the European Mouse Mutant Archive (EMMA, www.emmanet.org) will be one of the main repositories for the IMPC resource.
Here we report on the status of the new world wide program and present results showing proof of principle.

High resolution, quantitative mass spectrometry combines proteomics and genomics

Presenting Author: Matthias Mann

Max Planck Institute for Biochemistry, Munich, Germany

It would clearly be desirable to complement today's powerful DNA and RNA based methods with approaches that have a similar depth and precision for all cellular proteins. Here we discuss recent progress towards whole proteome identification and quantification using the latest advance in quantitative, high resolution mass spectrometry (MS) 1. The proteomes of model organisms can now be measured completely in just a few hours 2. The proteomes of human cell lines are not yet entirely mapped but coverage is approaching completion 3. Furthermore, MS-based proteomics has recently become sufficiently sensitive to investigate of relatively small sample amounts from paraffin embedded samples. This opens up for the study of distinct cell populations from in vivo sources. We will illustrate this by the analysis of cancer tissues and by a first pass atlas of protein expression in the mouse model.
Beyond expression proteomics, mass spectrometry can now determine protein interactors with exceeding specificity. The principle of this approach is to quantify the proteins binding to one or the other form of DNA, such as a binding motif and a control sequence. The method is generic and highly discriminating. Our laboratory is in the process of applying it to SNPs associated with a number of different diseases. This will be demonstrated with recent studies on type I diabetes4.

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3 Nagaraj, N. et al. Deep proteome and transcriptome mapping of a human cancer cell line. Mol Syst Biol 7, (2011).
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National Genome Research Network

Oral Presentation Abstracts

Symposium III

Genomics of Common Disease I

Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study

Presenting Author: André Reis

Anita Rauch (1,2)*, Dagmar Wieczorek (3)*, Elisabeth Graf (4)*, Thomas Wieland (4)*, Sabine Endele (2), Thomas Schwarzmayr (4), Beate Albrecht (3), Deborah Bartholdi (1), Jasmin Beygo (3), Nataliya Di Donato (5), Andreas Dufke (6), Kirsten Cremer (3), Maja Hempel (7), Denise Horn (8), Juliane Hoyer (2), Pascal Joset (1), Albrecht Röpke (9), Ute Moog (10), Angelika Riess (6), Christian T. Thiel (2), Andreas Tzschach (6), Antje Wiesener (2), Eva Wohlleber (11), Christiane Zweier (2), Arif B. Ekici (2), Alexander M. Zink (11), Andreas Rump (5), Christa Meisinger (12), Harald Grallert (13), Heinrich Sticht (14), Annette Schenck (15), Hartmut Engels (11), Gudrun Rappold (10), Evelin Schröck (5), Peter Wieacker (9), Olaf Riess (6), Thomas Meitinger (4,7), André Reis (2)*, Tim M. Strom (4,7)*
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The genetic cause of intellectual disability in most patients is unclear because of the absence of morphological clues, information about the position of such genes, and suitable screening methods. We used exome sequencing in parent-child trios to identify de novo variants in 51 individuals with sporadic non-syndromic intellectual disability from 10 centres in Germany and Switzerland of the German Mental Retardation Network (MRNET). 20 trios from the KORA Study were investigated as controls. We identified 87 de novo variants in the case group resulting in an exomic mutation rate of 1.71 per individual per generation. The corresponding figures in the control group were 24 de novo variants and 1.2 events per individual per generation. Most strikingly, a considerably higher number of individuals in the case group had loss-of-function variants (cases=20/51 vs. controls=2/20) indicating their contribution to disease etiology. Evaluation of the single variants revealed that 16 patients carried de novo variants in known ID genes with three recurrently mutated genes (STXBP1, SYNGAP1, SCN2A). Focusing only on loss-of-function variants, we considered at least 6 mutations in 6 novel genes to be disease causing. We further identified several missense alterations with potential pathogenicity. Interestingly, our study revealed no evidence for a sizable contribution of autosomal recessive inheritance.
After exclusion of copy-number variants, de-novo point mutations and small indels are associated with severe, sporadic non-syndromic intellectual disability, accounting for 45–55% of patients with high locus heterogeneity. Autosomal recessive inheritance seems to contribute little in the outbred population investigated. The large number of de-novo variants in known intellectual disability genes is only partially attributable to known non-specific phenotypes. Several patients did not meet the expected syndromic manifestation, suggesting a strong bias in present clinical syndrome descriptions.

Pre-, peri- and postnatal Stress in human and non-human off-spring: a convergent approach to study genetic and epigenetic impact on depression

Presenting Author: Vanessa Nieratschker

Vanessa Nieratschker (1), Maria Gilles (2), Renaud Massart (3), Alessio Luoni (4); Michael Deuschle (2); Manfred Laucht (5); Marcella Rietschel (1); Peter Gass (6); Isabell Wolf (2); Verena Peus (2); Marc Sütterlin (7); Marco Riva (4); Francesca Cirulli (8); Markus Nöthen (9); Sven Cichon (10); Moshe Szyf (3)

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Exposure to early life stress (ELS) has been associated with an increased risk for psychiatric diseases, especially depression later in life. Given the high prevalence of stress-related conditions in pregnancy, the role of ELS on the offspring's health in adulthood is an area urgently requiring further research. Animal models have implicated epigenetic regulation of gene expression as mediators of this effect. In our study, we apply an integrated cross-species approach that covers different tissues, different time points as well as different stressors.

We performed whole-genome methylation analyses in hematopoietic stem cells derived from cord blood of neonates exposed to extremely low vs. high levels of prenatal stress. The human data were analyzed using a convergent approach including data resulting from non-human primates and rodents.

We found that ELS leads to differential DNA methylation in hematopoietic stem cells. In all, we found 3405 distinct gene promoters whose normalized intensities were significantly associated with ELS. By comparing the human data with the animal data, we identified many overlapping genes differentially methylated across species and time points. One gene was differentially methylated in all species, tissues and time points analyzed. In addition, analyzing GWAS data, we found genetic evidence for an involvement of this gene in depression.

Our results support the hypothesis that the response to ELS is system-wide and genome-wide and persists into adulthood in animals and presumably also in humans. Our data also points to the feasibility of studying the impact of the social environment on DNA methylation in peripheral tissues. Applying genome-wide methylation analysis on peripheral tissue could therefore have a great potential for the prediction of future health outcomes and identification of risk groups.

Host-microbe interactions shape genetic risk for inflammatory bowel disease

Presenting Author: Andre Franke

Andre Franke (1), David Ellinghaus (2), Stefan Schreiber (3) on behalf of the International IBD Genetics Consortium

(1) Institute of Clinical Molecular Biology
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(3) Hospital Schleswig-Holstein, Kiel, Germany

Crohn's disease and ulcerative colitis are the two common forms of inflammatory bowel disease. Genome-wide association scans have implicated multiple susceptibility genes for these disorders, but an integrated view of the underlying biological pathways is lacking. We sought to expand knowledge of relevant pathways by undertaking a meta-analysis of Crohn's disease and ulcerative colitis genome-wide association scans, with validation of significant findings in more than 75,000 cases and controls employing the custom "Immunochip". We identify 71 new loci resulting in a total of 163 loci with genome-wide significance. Most loci contribute to both phenotypes and both directional and balancing selection effects are evident. We observe striking overlap between susceptibility loci for inflammatory bowel disease and mycobacterial infection. Gene expression network analysis emphasizes this relationship, with pathways shared between host responses to mycobacteria and those predisposing to inflammatory bowel disease. Collectively, these findings shed light on the host-microbe interactions underlying this archetypal complex disease. This study is in press in Nature.

Mapping the Functional Epigenome of Ulcerative Colitis in Monozygotic Twins

Presenting Author: Robert Häsler

Robert Häsler (1), Zhe Feng (1), Liselotte Bäckdahl (2), Martina E. Spehlmann (1), Andre Franke (1), Andrew Teschendorff (2), Vardhman K. Rakyan (3), Thomas A. Down (4), Gareth A. Wilson (2), Andrew Feber (2), Stephan Beck (2), Stefan Schreiber (1), Philip Rosenstiel (1)

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The genetic background only partially explains the susceptibility, manifestation and progression of ulcerative colitis (UC), one of the two major subforms of inflammatory bowel disease. In addition to genetics, environmental factors may influence disease mechanisms by modulating the epigenetic landscape. To test this hypothesis, we analyzed DNA methylation (DNAm), a fundamental mechanism of epigenetic long-term modulation of gene expression.

The presented study is based on a three-layer epigenomewide association study (EWAS) employing intestinal biopsies from monozygotic twins, discordant for ulcerative colitis. The three layers are represented by i) genome wide transcriptome data (mRNA, Affymetrix UG 133 Plus 2.0); ii) genome wide data on methylation variable positions (MVPs, Illumina 27k Infinium Bead Array); and iii) genome wide data on differentially methylated regions (DMRs, MeDIP-Chip Nimblegen, custom 385k Tiling Array). Validation of identified mRNAs, MVPs and DMRs was carried out by real-time PCR and bisulfite-pyrosequencing in a larger patient group (n=185).

Our analysis identified 61 disease-associated loci where modulated DNAm is neighbored by a differentially expressed transcript. Interestingly, none of the candidate risk loci were previously identified by GWAS. Several of the presented candidate loci have been functionally liked to inflammatory processes e.g. complement factor CFI, the serine protease inhibitor SPINK4 and the adhesion molecule THY1/CD90. The inclusion of disease specificity controls excludes non-disease inflammation as cause of the observed modulation of DNAm

In conclusion, our study represents the first replicated EWAS of UC linked to transcriptome profiles in disease tissue and emphasizes the strength of such an analysis in understanding unexplained disease risk and molecular events of disease manifestation.

Childhood ADHD and obesity: evidence for a common genetic link

Presenting Author: Anke Hinney

Özgür Albayrak MD (1), Carolin Pütter MSc (2), Anna-Lena Volckmar (1), Sven Cichon PhD (3,4), Per Hoffmann PhD (4), Markus M. Nöthen MD (4), Karl-Heinz Jöckel PhD (2,5), Stefan Schreiber MD (6), H-Erich Wichmann PhD (7), Stephen V. Faraone PhD (8,9), Benjamin M. Neale PhD (9,10,11) , Collaborators: Psychiatric GWAS Consortium: ADHD subgroup (9), Beate Herpertz-Dahlmann MD (12), Gerd Lehmkuhl MD (13), Judith Sinzig MD (13,14), Tobias J Renner MD (15), Marcel Romanos MD (15,16), Andreas Warnke MD (15), Klaus-Peter Lesch MD (17), Andreas Reif MD (17), Benno G Schimmelmann, MD (18), André Scherag PhD (2), Johannes Hebebrand MD (1), Anke Hinney PhD (1)

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Background Epidemiological and clinical studies show that children and adolescents with ADHD have higher rates of obesity than children without ADHD. Several case reports of children with severe obesity and co-morbid ADHD indicate e.g. a role for genetic mutations in the melanocortin-receptor 4 gene (MC4R) (Albayrak et al., 2011). Gene variants predisposing to obesity potentially overlap with those relevant for ADHD.

Methods We screened 32 known obesity risk alleles (Speliotes et al., 2010) from a genome-wide association study (GWAS) for ADHD based on 495 young German patients (Illumina; Human660W-Quadv1 BeadArrays) and 1,300 population-based controls (Illumina;HumanHap550v3 BeadArray). We also performed in silico analyses of the SNPs in a large ADHD meta-analysis comprising 2,064 trios, 896 cases, and 2,455 controls (Neale et al., 2010). In addition, we explored the obesity risk alleles with regard to their quantitative effects on inattention and hyperactivity/impulsivity in both samples.

Results In the German sample rs206936 in the NUDT3 gene was associated with ADHD risk (OR: 1.39; p=3.4x10-4; p=0.01 upon correction for 32 tests). In silico analysis of the meta-analysis data revealed the major allele of rs6497416 in the intronic region of the GPRC5B gene (p=7.2 x 10-4; pcorr=0.02) as risk allele for ADHD. The risk allele is in high linkage disequilibrium with the obesity risk allele at rs12444979. Exploratorily, BMI SNPs in NUDT3 and GNPDA2 were nominally associated with inattention (p<0.05) in the German sample, whereas markers in MAP2K5 and CADM2 were nominally associated with hyperactivity and SNPs in GPRC5B with the combined phenotype.

Discussion Our results suggest that BMI rsik alleles at the NUDT3 and at the GPRC5B locus confer an increased risk for ADHD. GPRC5B is homologous to genes of the metabotropic glutamate receptor family, which have recently been implicated in the etiology of ADHD (Elia et al., 2010, 2012).

An integrated catalogue of genome, methylome and gene expression variations in colon cancer

Presenting Author: Ralf Herwig (1)

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Advances in high-throughput sequencing enable the deep characterization of cancer genomes at various levels of molecular information. We have analyzed genome-wide methylation and gene expression of colon cancer patients using methylated DNA immunoprecipitation followed by sequencing (MeDIP-seq) and RNA-seq. We identified targeted hypermethylation in promoters of tumour-relevant genes and their subsequent down-regulation. Furthermore, a major outcome of this work was the development of the MEDIPS software, the first comprehensive approach for normalization and differential analysis of MeDIP-seq data. MEDIPS generates differential methylation candidate regions with high accuracy (>0.9) [1]. Additionally, since we observed a fundamental influence of the genetic alterations on both methylation and gene expression, we have added high coverage whole-genome sequencing (80-100x) that allowed a deep characterization of somatic sequence variation as well as allele-specific analysis of methylation and gene expression.
In our presentation we describe the multiple complex landscapes of patient tumors (genome, methylome and transcriptome) and give an integrated view of the different bits of genomic information with selected examples. Furthermore, we describe an approach for the computation of network modules from interaction data [2] that allows combining the genomic sequencing data with molecular networks in order to better understand the functional consequences of sequence variation.

[1] Chavez, L., Jozefczuk, J., Grimm, C., Dietrich, J., Timmermann, B., Lehrach, H., Herwig, R.(*), Adjaye, J.(*) (*) equal contribution (2010) Computational analysis of genome-wide DNA-methylation during the differentiation of human embryonic stem cells along the endodermal lineage. Genome Res, 20:1441-1450.

[2] Kamburov, A., Pentchev, K., Galicka, H., Wierling, C. K., Lehrach, H., Herwig, R. (2011) ConsensusPathDB - towards a more complete picture of cell biology. Nucleic Acids Res, 39(1):712-717.

Evolutionary Medicine and Molecular Medicine: Synergistic Siblings

Presenting Author: Randolph M. Nesse

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Evolutionary medicine is the field that applies the principles of evolutionary biology to the problems of medicine and public health. There should be no need for such a field— all health professions should rely on evolution as a basic medical science. However, accidents of history have left evolution out of most medical curricula, thus offering an opportunity to bring a neglected medical science to bear on urgent human problems.

George Williams and I encouraged scientists to ask new questions about why natural selection left bodies with traits such as a narrow birth canal and apparently poorly regulated immune responses that make us vulnerable to diseases. Such questions had not been taken seriously because it was not obvious how natural selection could explain maladaptations. However, our list of six possible reasons for traits that leave bodies vulnerable to disease has proved robust in encouraging research on evolutionary explanations of disease to complement proximate explanations. It has also encouraged close attention to how diseases arise from interactions of genetic variations with novel environments, and to how natural selection shaped mechanisms that regulate the expression of facultative defenses such as pain, fever, anxiety, and inflammation.

While seeking evolutionary explanations for disease vulnerability has spurred great interest, this is only one part of a larger fast-growing field. For instance, evolution has long provided a foundation for population genetics, but some major advances in evolutionary biology are only now being applied in molecular medicine. They include the need for both proximate and evolutionary explanations, kin selection, evolutionary models for cooperation, competition among alleles, co-evolution, and new strategies for tracing phylogenies and identifying signals of selection. Phylogenetic methods have also long been useful in medicine, and they too are being revised to take advantage of new advances in genomics. Such methods have especially clinically relevant for studying antibiotic resistance, virulence evolution, and evolution taking place in neoplasms.

Collaborations between evolutionary biologists and medical geneticists offer the promise of major advances. Support for strategies that encourage such collaborations will improve human health.

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Oral Presentation Abstracts

Symposium IV

Genomics of Common Disease II

Innovations in translational research – Successes of NGFN

Presenting Author: Hugo A. Katus

Hugo A. Katus, Tanja Weis, Benjamin Meder

Heidelberg University Hospital, Germany

Genomic research of human diseases must lead to a better care of patients. Here we discuss our strategies to improve the diagnosis of heart failure and heart failure risk by utilizing DNA, microRNA, metabolites and proteins as biomarkers and demonstrate their impact on clinical care and patient outcome.
DNA: Many cardiomyopathies are caused by mutations of cardiac constituents. Since the genotype can determine outcome (e.g. LaminA and sudden cardiac death), we have embarked on the development of highly efficient tools for DNA-sequencing and data interpretation, suitable for a routine clinical application.
MicroRNAs: The analysis of whole blood microRNA profiles provides comprehensive information to the disease process not only caused by alterations in cardiac tissue but also of the (mal)-adaptive changes of the entire organism. By a combined analysis of dysregulated microRNAs, some with yet unknown biological function, one is able to detect myocardial infarction even before the rise of established cardiac biomarkers and identify novel markers for heart failure.
Metabolites: The unbiased profiling of metabolites in blood may allow an integrated assessment of the functional consequences and severity of diseases. We performed mass spectrometry based high-throughput profiling of metabolites in blood of patients with cardiomyopathies. Markedly, distinct metabolite profiles were observed even in preclinical heart failure providing novel clues as to the disease mechanisms and pathways.
Proteins: We have developed the troponin T assay which has revolutionized the care of patients with suspected heart diseases. By the high sensitivity assay it is now possible to detect minute myocardial cell injury of multiple causes and assess the risk of presumably healthy populations.
Thus, the integrated use of biomarkers of different classes and functions improved patient, saving more than 100.000 lives annually.

Association of X chromosomal variants with coronary heart disease: Results from a meta-analysis

Presenting Author: Christina Loley (1, 2)

Heribert Schunkert (3), Jeanette Erdmann (2), Inke R. König (1)

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In many genome-wide association studies, the focus is on the analysis of autosomal markers, and regions on the X chromosome are often neglected. The likely reason for this is that, up to now, no standard statistical method exists to adequately deal with the specific features of X chromosomal data. Thus, potentially important genetic information remains undetected. Especially, when the disease of interest is known to show gender-specific effects, the sex chromosomes are likely to play an important role for the inheritance of the disease.

In this project, we analyzed X chromosomal associations with coronary heart disease, where gender specific effects are well described. The analysis was based on data from the CARDIoGRAM consortium (Schunkert et al. 2011) with genotypes imputed on the 1000 genomes data. To perform study-wise association analysis, logistic regression models were chosen that account for the specific data structure of chromosome X, including the phenomenon of inactivation of one female X chromosome. In a second step, a meta-analysis of all participating study cohorts was calculated using fixed and random effect models. To reduce heterogeneity between studies, an outlier analysis was applied and studies with extreme effect estimates were excluded.

We will present results from 12 international study cohorts including nearly 15,000 cases with coronary artery disease (CAD) or myocardial infarction and nearly 15,000 independent controls. We have discovered several regions displaying chromosome-wide significant association with CAD. Most of these regions are rich of genes that may give a hint at the underlying genetic mechanisms.

Schunkert H, König IR, Kathiresan S et al. Nat Genet , 2011, 43, 333-8

Toward a novel genomics-based taxonomy of lung cancer: The Clinical Lung Cancer Genome Project

Presenting Author: Martin Peifer

Martin Peifer (1), Roman K. Thomas (1)

(1) Department of Translational Genomics, Center of Integrated Oncology Köln - Bonn, University of Köln

To date, commonly used lung cancer classification systems are largely based on histopathology. However, recent lung cancer genome analysis efforts revealed distinct patterns of genetic alterations that correlate not only with histology but also with the clinical outcome of patients. Furthermore, already today individual treatment decisions are based on individual genetic alterations (e.g. EGFR-TKIs in EGFR-mutant patients, ALK inhibition in EML4-ALK fusion-positive patients). We therefore launched The Clinical Lung Cancer Genome Project Initiative with the goal of establishing a novel, molecular genetics taxonomy of lung cancer based on the deep genomics analysis of over 1,000 primary lung cancers, on the association of cancer genotypes and clinical outcome and on a comprehensive pathology review by a panel of expert lung cancer pathologists. We have collected over 1,800 clinically annotated fresh-frozen lung cancer tumors of all histological subtypes and genomics analyses have been conducted on more than 1,000 of these. In addition to known molecular subtypes, the large size of our sample set and the resolution of our analyses enabled the identification of genetically defined subsets that segregated with overall survival and other clinical parameters. Furthermore, we have identified novel genetic alterations that are therapeutically tractable, including FGFR1 amplifications in squamous-cell lung cancer, a tumor type lacking specific therapeutic targets so far. In addition, the broad representation of many rare lung cancer subtypes afforded detection of novel gene fusions as well whole-exome sequencing of small cell lung cancer. In this highly mutated lung cancer subtype, we found novel recurrently mutated genes, the relevance of which was validated in functional cellular assays. In summary, our projects offers unique opportunities to study the genomes of lung cancer and to establish a new lung cancer taxonomy based on genomics, histopathology and clinical outcome.

Aberrant Self-Renewal and Quiescence Contribute to Glioblastoma Aggressiveness

Presenting Author: Christel Herold-Mende

Christel Herold-Mende (1), Benito Campos (1), Tilman Schneider (1), Zoltan Gal (1), Christopher Sliwinski (1), Aline Baader (1), Andreas Unterberg (1)

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Objective: Stem-like tumor cells endowed with enhanced self-renewal capacity are believed to drive tumor growth in malignant gliomas. So far a variety of surrogate markers has been proposed to characterize and enrich these cells emphasizing the need of devising new isolation methods based on common functional and phenotypic criteria.
Methods: In this study we made use of a collagen-based self-renewal assay, to screen for clonogenic cell subpopulation in malignant gliomas. Cells were profiled using a gene expression chip and tested for tumor formation capacity in an orthotopic mouse model. Label retention was used to detect quiescent tumor cells.
Results: In a panel of glioblastoma cell lines (n=23) we identified several cell lines enriched for cells with enhanced self renewal capacity. These cell lines were capable of matrix-independent growth and formed fast-growing, orthotopic tumors in mice. Employing isolation and re-plating techniques, we could further show that these cells invariably re-established a cellular hierarchy through a series of asymmetric cell divisions. However, the ratio of symmetric to asymmetric cell divisions seemed to be pathologically increased and was linked to idiosyncratic transcriptomal changes as well as to poor overall survival of corresponding patients. Finally, through label-retention experiments we further identified a subpopulation of quiescent and chemo-resistant cells, which retained the ability to reinitiate growth of secondary cell clones and thus, might play a role in tumor recurrence after therapy.
Conclusions: Altogether, our results suggest tumor quiescence and aberrant proliferations influence the aggressiveness of glioblastoma.

National Genome Research Network

Oral Presentation Abstracts

Symposium V

Functional Genomics

Genome sequencing of childhood medulloblastoma brain tumors links chromothripsis with TP53 mutations

Presenting Author: Jan Korbel

Tobias Rausch (1), David Jones (2), Marc Zapatka (2), Adrian Stütz (1), Peter Lichter (2), Stefan Pfister (2), Jan Korbel (1)

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Somatic structural variations typically occur progressively during tumor development. Recent findings, however, suggest an alternative mechanism, involving chromosome shattering and reshuffling ('chromothripsis'), the underlying mechanistic basis of which is unknown. In the context of the ICGC Pediatric Brain Tumor Project, whole-genome sequencing of a Sonic-Hedgehog medulloblastoma (SHH-MB) brain tumor from a patient with a germline TP53 mutation (Li-Fraumeni syndrome) revealed massive, complex rearrangements resulting from chromothripsis. Integrating TP53 status with genomic rearrangement data in additional medulloblastomas revealed a striking association between TP53 mutation and chromothripsis in SHH-MBs. Unexpectedly, five seemingly sporadic SHH-MB patients with chromothripsis harbored TP53 germline mutations, findings that are relevant for clinical management and which offer initial mechanistic insights: i.e., owing to their germline status those TP53 mutations must have preceded chromothripsis, which implies a causal connection, whereby mutant p53 may induce chromothripsis or facilitate cell survival following massive SR occurrence. Analysis of additional tumor entities substantiated a link between TP53 mutation and chromothripsis, beyond general genomic instability. Among these, we observed a strong association between somatic TP53 mutations and chromothripsis in acute myeloid leukemia. These findings implicate p53 in the initiation of, or cellular reaction to, chromothripsis – a novel role for the 'guardian of the genome'. Along with these recently published findings [1] we will present new analyses that further substantiate the involvement of chromosome shattering in the massive rearrangements associated with chromothripsis.

Reference:
[1] Rausch et al., Cell 2012, 148:59-71.

Secretion of Wnts is required for Wnt/ß-catenin pathway activity in colorectal cancer (CRC) despite APC or ß-catenin mutations

Presenting Author: Gerrit Erdmann

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Aberrant activation of Wnt signaling through mutations in APC or ß-catenin are directly linked to colorectal cancer (CRC), the third most common cancer worldwide. While Wnt signaling is required for maintenance of the intestinal homeostasis and its misregulation is a starting point for additional mutations ultimately leading to the formation of colorectal carcinomas. Yet, the molecular mechanisms that govern aberrant activation caused by mutations in APC or beta-catenin are not fully understood.
As part of NGFN IG on colorectal cancer, we investigated how these mutations influence the structure of the Wnt/ß-catenin pathway by combining depletion of key pathway components by RNAi with RNAseq and subsequently nested effects modeling (NEM). Based on the resulting transcriptome profiles, NEM indicated that blockage of Wnt secretion impairs the expression of ß-catenin dependent transcriptional targets despite activating mutations in ß-catenin. We demonstrated that CRC cell lines remain dependent on Evi/Wls mediated Wnt secretion despite mutations in APC or ß-catenin. Furthermore, we found that EVI/WLS and canonical Wnts are often highly expressed in human tumour samples correlating to poor outcome.
Mechanistically, we observed that various truncated forms of APC retain ß-catenin binding ability and are still able to recruit additional components of the destruction complex regulating ß-catenin stability. Thus, mutations in APC or ß-catenin are not dominant over upstream regulation but sensitize cells to canonical Wnt ligands. This finding implies novel strategies for therapeutic intervention in CRC upstream of APC or ß-catenin.

LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression.

Presenting Author: Sven Lindner

Sven Lindner (1), Jan J. Molenaar (2), Pieter Mestdagh (3), Theresa Thor (1), Annika Sprüssel (1), Huib N. Caron (2), Rogier Versteeg (2), Alexander Schramm (1), Angelika Eggert (1), Johannes H. Schulte (1)

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Background: Overexpression of LIN28b has been reported in neuroblastomas (NB) and other malignancies. LIN28b is known to repress let-7 miRNAs, which target MYCN, but LIN28b tumor-initiating capacity and oncogenicity has not been investigated in vivo. We overexpressed murine Lin28b in the neural crest to determine if Lin28b can drive neuroblastomagenesis.
Methods: Murine Lin28b was conditionally expressed by knock-in of the CAG-LSL-Lin28b-IRES-Luc vector into the ROSA26 locus. Mice were crossbred with DBH-iCre mice to target expression to the neural crest. Arising tumors were characterized using histology, immunohistochemistry, PCR and western blotting, and maintained via serial transplantation.
Results: Abdominal tumors developed in 6 of 16 DBHiCre;LSL-Lin28b transgenic mice at 36-56 days of age. Autopsy revealed uni- or bilateral adrenal tumors, reflecting the most frequent localization of human NB. Thoracic tumors and tumors originating from the superior cervical or celiac ganglia were observed. Tumors consisted of small round blue cells and expressed the NB markers, DBH, TH and Phox2b. The macroscopic tumor appearance, primary tumor sites, tumor histology and marker gene expression confirmed these tumors as NB. Lin28b and MYCN proteins were expressed in all tumors and let-7 miRNAs were downregulated. Successful serial transplantation in immunocompromized mice supported that the primary tumors were fully transformed malignant tumors. Treating those mice with the bromodomain inhibitor JQ1 resulted in enhanced cell death and decreased cell proliferation.
Conclusions: We demonstrate that Lin28b can drive NB in mice, supporting LIN28b as an important oncogene for NB. Our results suggest that, similar to human NB, MYCN is at least in part induced via downregulation of let-7 miRNAs by Lin28b overexpression in mice. Therapeutic approaches aimed at inhibiting Lin28b-let-7 interaction may be a useful therapeutic approach.

Human BCL2-associated athanogene 3 (BAG3) mutations lead to dilated cardiomyopathy in zebrafish

Presenting Author: Sören Westphal

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Dilated cardiomyopathy (DCM) affects 2.5 per mill adults and long term survival is devastating poor. Recent GWAS suggest SNPs in the Bag3 gene to be involved in the pathogenesis of DCM but the biological significance of this finding remains to be elucidated. The zebrafish is emerging as an important vertebrate model organism in the field of functional genomics and is established as a tool to address these questions. We identified the ortholog of human Bag3 in zebrafish and conducted Bag3 knock-down experiments by injecting morpholino (MO)-modified antisense oligonucleotides into one-cell–stage zebrafish embryos. MO-injected embryos showed normal heart morphogenesis during the first 24 hours post fertilization. Subsequently, morphants developed heart failure with cardiac-chamber dilation, reduced systolic function, and precardial blood congestion. Interestingly, this phenotype could be prevented by co-injection of human wt Bag3 mRNA suggesting that biological function seems to be conserved. To evaluate the impact of the identified mutations on Bag3 function, we expressed either wt or mutagenized (mut) human Bag3 containing mutations corresponding to those found in the GWA study. Noteworthy, we used constructs containing wt or mut Bag3 under the control of the cardiac mlc2 promotor to express or gene of interest in a tissue specific manner. After injection of plasmids carrying either the E455K or V468M mutation embryos developed a DCM phenotype indistinguishable from MO-Bag3–injected fish. Notably, injection of an equal amount of plasmid carrying the wt human Bag3 sequence has no effect. For selected mutations we currently generate inducible (TetOn/TetOff system), transient, and stable heart-specific transgenic zebrafish lines to assay their impact on adult heart muscle structure and function. In summary, these findings confirm the disease-causing nature of the identified human Bag3 mutations and show that mutant Bag3 proteins act in a dominant-negative manner.

Cyp17a1 deficient mice display increased body weight, visceral/subcutaneous fat deposition and altered lipid metabolism

Presenting Author: Zouhair Aherrahrou

Zouhair Aherrahrou (1,3), Redouane Aherrahrou (1,3), Kjestine Schmidt (2,3), Thorsten Kessler (3,5), Jaafar Al-Hasani (1,3), Jennifer Freyer (1,3), Natalia Alenina (4), Michael Bader (3,4), Cor de Wit (2,3), Heribert Schunkert (3,5) & Jeanette Erdmann (1,3)

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Cyp17a1 belongs to the cytochrome P450 enzyme complex 17alpha-hydroxylase/C17, 20-lyase. Genome-wide association studies (GWAS) revealed genetic variants within the Cyp17a1 locus to be associated with coronary artery diseases (CAD) as well as hypertension. However underlying pathomechanisms are completely unknown.
In this study we aimed to investigate the functional role of Cyp17a1 by generating a mouse deficient for this protein.
Cyp17a1-deficient embryonic stem cells containing an insertion of a neomycin/IRES/ß-Gal selection cassette between exon 3 and 4 and two loxP sites flanking exon 4 to 6 were purchased from EUCOMM. After verification of the knockout using X-Gal staining and PCR, these cells were used to generate mice deficient for Cyp17a1 (Cyp17a1-/-). Cyp17a1-/- mice were viable and were phenotypically characterized at the age of 4-6 months for behaviour, body weight, and lipid metabolism. In addition, arterial blood pressure and heart rate were measured by telemetry in conscious mice.
The frequencies of genotypes in the progeny after mating heterozygous animals approximated the Mendelian ratios. All Cyp17a1-/- mice (n=22) presented with an apparent female phenotype but of these 55% carried a Y chromosome. Cyp17a1-/- mice had increased body weight. Interestingly, this increase in body weight was associated with enhanced visceral and subcutaneous fat deposits and altered lipid metabolism. Heart rate, systolic and diastolic blood pressures were not different between genotypes (n=4 each).
Expectedly, Cyp17a1 deficiency interfered with sexual differentiation. In addition, our data demonstrate for the first time a key role of Cyp17a1 in lipid metabolism and fat deposition leading to obesity. It remains to be explored if these alterations translate to enhanced atherosclerosis and association with CAD.

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Oral Presentation Abstracts

Symposium VI

Personalized Medicine

Systems approaches to Parkinson´s disease

Presenting Author: Rudi Balling

Luxembourg Centre for Systems Biomedicine, Luxembourg

Parkinson´s disease is one of the major neurodegenerative diseases and primarily due to the loss of dopaminergic neurons in the substantia nigra. Pathogenesis of PD is not well understood, however it is thought to be multi-factorial and age-related with many genetic and environmental factors involved. In order to capture the rapidly increasing information and inter-relationships between different factors contributing to PD we are in the process of establishing a SBGN-based “PD-disease map”. The map captures and visualizes all major molecular pathways involved in PD pathogenesis and can serve as a resource for further computational analyses and as a platform for community level collaborations. We have also begun to study the role of neuroinflammation in the development of neurodegeneration. For this purpose we analyzed the metabolomic response of microglia cells to inflammatory stress and identified a new metabolite and pathway involved in metabolic mediated immunity.

A promising therapy strategy for PDAC: MEK1/2 inhibition with the novel chemotherapeutic drug BAY 86-9766 (RDEA119)

Presenting Author: Nicole Teichmann (1)

Marija Trajkovic-Arsic (1), Irina Heid (2), Arne Scholz (3), Roland M. Schmid (1), Rickmer Braren (2), Jens T. Siveke (1)

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To date, pancreatic ductal adenocarcinoma (PDAC) remains a devastating and deadly disease due to unavailable biomarker for early diagnosis and high intrinsic resistance to any therapeutic intervention. Thus, novel effective agents as well as better predictive models are urgently needed.
In this preclinical study, we evaluated a novel, highly selective MEK1/2 inhibitor BAY 86-9766 for the first time in an endogenous mouse model of PDAC.
Using a Cre/loxP approach, we generated mice with pancreas specific activation of Kras and concomitant deletion of p53 (Ptf1a+/Cre, Kras+/LSL-G12D, p53loxP/loxP; CKP). Those mice typically develop lethal invasive PDAC within 8 weeks of age. Non-invasive MRI was used to track tumor progression during therapy. Primary tumor cells isolated from vehicle and BAY 86-9766 treated animals were used for molecular and functional assays to identify possible resistance mechanisms for the development of sequential therapy strategies.
Treatment of various human and mouse pancreatic cell lines with BAY 86-9766 resulted in a dose-dependent inhibition of MEK1/2 as well as induction of apoptosis via upregulation of caspase 3/7 activity and the proapoptotic protein Bim. In vivo, BAY 86-9766 prolonged the survival of CKP mice significantly with a median survival advantage of 20 days. Moreover, a dramatic decrease of the tumour load was observed already after 1 week of treatment. In most animals, tumors relapsed typically after 3 weeks of therapy revealing a notable change in morphological appearance. Interestingly, molecular analysis of primary mouse pancreatic tumor cell lines from recurrent BAY 86-9766 treated PDACs identified an epithelial-mesenchymal transition (EMT) phenotype as one resistance mechanism, which was also evident via histological analysis. Moreover long-term treated, BAY 86-9766 resistant cells manifested an upregulation of the driving oncogene Kras as well as the AKT-mTOR signaling cascade, which could be involved in triggering the EMT.

Haplotype-Resolving Multiple Human Genomes: Key to Personalized Medicine and Genome Biology

Presenting Author: Margret R. Hoehe (1)

Margret R. Hoehe (1), Eun-Kyung Suk (1), Gayle K. McEwen (1), Thomas Huebsch (1)

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Human individuals are diploid by nature, have two sets of chromosomes, one from each parent. With about four million variants per individual, any two homologous chromosomes or genes can be genetically very different. Thus, independent determination of both haplotype sequences of an individual genome is essential to relate genetic variation to genome function, phenotype and disease. To this end, we have developed a novel fosmid pool-based 'next generation sequencing' approach to haplotype-resolve whole genomes and generated the most completely haplotype-resolved individual genome to date, 'Max Planck One', underscoring the importance of phasing (featured in Nature Methods Special 'Methods of the Year 2011'). We have moreover demonstrated the power of our approach by haplotype-resolving HapMap trio child NA12878, phasing 20% more of SNPs compared to 1000G trio sequencing, particularly also in disease-related regions. We have now haplotype-resolved a total of 14 genomes from a representative German population cohort (PopGen), an unprecedented number, and comparatively evaluated 57CEU up to 372EUR phased genomes from 1000G. In summary, we present a first global view of molecular haplotype and diplotype diversity existent across nearly 400 European genomes. An exorbitant diversity of individually different molecular gene forms is evident, with about 4 million molecular haplotypes being unique in 372 genomes, over 10,000 per individual. Only about 14% of all genes were found to have one major form. At the protein level, multiple individual proteomes, defined by the totals of all genes that encode two different molecular forms, were found to converge upon an ever more common diplotypic proteome. To summarize, we provide first insights into the variation of molecular haplotype and diplotype structures within and between genomes, essential knowledge to develop valid approaches to individualized medicine, while advancing diploid genome biology and the elucidation of disease.

The Risk of Myoacardial Infarction is increased by Digenic Mutation in GUCY1A3 and CCT7 - identified by exome sequencing in an extended family.

Presenting Author: Jeanette Erdmann

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Myocardial infarction (MI) is a life-threatening disease, which results from sudden atherothrombotic occlusion of a coronary artery. Most cases of MI occur sporadically, but sometimes the disease clusters in families. Understanding the genetic basis of MI in affected families may illuminate its pathobiology. Here, we show in an extended MI family that heterozygous mutations in two functionally related genes, GUCY1A3 (p.Leu163Phefs*24) and CCT7 (p.Ser525Leu), segregate with MI. Out of 15 affected family members available for genetic analysis, 7 and 11 carried GUCY1A3 and/or CCT7 mutations, respectively (two-locus maximum LOD score of 5.68). Moreover, studying 28,733 MI or coronary artery disease (CAD) cases and 75,028 controls by genome-wide association analysis we identified an intronic SNP in GUCY1A3 as associated with MI/CAD (P <1.74x10-8 for rs7692387). GUCY1A3 encodes the a1-subunit of soluble guanylyl cyclase (sGC), while CCT7 encodes CCT-eta, a member of the chaperonin containing TCP1 complex (TRiC/CCT), which, among other functions, stabilizes sGC. Upon stimulation with nitric oxide (NO) the enzyme generates cGMP which induces vasodilation and inhibits platelet activation. We demonstrate in vitro that both GUCY1A3 and CCT7 mutations severely impair sGC activity. Moreover, platelets from digenic mutation carriers contained less sGC protein and consequently reduced NO-induced cGMP formation. Mice deficient for the a1-subunit sGC protein displayed accelerated thrombus formation in the microcirculation upon local trauma. Starting with a severely affected family, we have identified a link, possibly through accelerated thrombus formation, between impaired sGC dependent NO signaling and MI risk which may also relate to more common forms of the disease. Reversing this defect could provide a novel therapeutic target for reducing risk of MI.

High-throughput cell-based assays identify Placenta-specific 8 (Plac8; Onzin) as a key regulator of proliferation and survival in pancreatic cancer cells

Presenting Author: Brajesh P. Kaistha

Brajesh P. Kaistha(1), Ying Chen(1,3), Melanie Sauer(1), Harald Schmidt(1), Ramona Kreider(1), Jan Sperveslage(2), Bence Sipos(2), Thomas Gress(1) and Malte Buchholz(1)

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As part of the PaCaNet project within the NGFN program, we have performed highly parallelized functional assays to identify novel genes with central pathophysiological roles in pancreatic cancer. Functional effects of overexpression and knockdown of 80 pre-selected candidate genes in cancer and control cells were examined using reverse transfection microarrays. Genes producing significant and reproducable effects were selected for further in-depth characterization.
Among these was Placenta-specific 8 (Plac8; Onzin). The physiological function of Plac8 is unknown, and it has previously not been implicated in cancer. Plac8 expression is absent in normal pancreas as well as chronically inflamed pancreas tissue, but is strongly expressed in 70 % of human PDACs. Interestingly, this pattern of ectopic expression is mirrored in transgenic mouse models of PDAC. In vitro assays showed strong impairment of cell proliferation and viability following down-regulation of Plac8 expression in all pancreatic cancer cell lines tested. Western blot and flow cytometry assays did not demonstrate any apoptotic activity, and flow cytometry analyses confirmed that growth attenuation was primarily due to a block in cell cycle progression. Intriguingly, classical checkpoint mechanisms (p21, p53, Chk1, Cdc25A) were not activated, thereby suggesting involvement of unknown mechanisms. This was further supported by confocal laser scanning microscopy analyses, which demonstrated specific interaction of Plac8 with structures at the cytoplasmic side of the plasma membrane, thus rendering direct regulation of cell cycle checkpoint mechanisms unlikely. SILAC (stable isotope labeling)-enabled mass spectrometry as well as reverse phase protein microarrays (RPMAs) were used to identify protein interaction partners of Plac8 and pathways affected by Plac8 knockdown, respectively. Generation of inducible shRNA clones as well as Plac8 transgenic mice for further in vivo experiments is in progress.

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Poster Presentation Abstracts

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Poster Presentation Abstracts

Symposium III

Genomics of Common Disease I

Endocannabinoid long-term depression is a common molecular mechanism for drug and natural reward-seeking

Presenting Author: Ainhoa Bilbao

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Throughout the animal kingdom, reward-seeking is vital for individuals and necessary for the survival of species. However, the precise molecular and physiological bases of reward-seeking behavior remain largely unknown. Here we show that mice with a conditional knockdown of mGluR5 in dopamine D1 receptor-expressing neurons (D1miRmGluR5) do not show drug- or natural reward-seeking behavior and lack endocannabinoid (eCB)-mediated long-term depression (LTD) in the mesocorticolimbic reward system. Using pharmacological enhancement of 2-arachidonoylglycerol (2-AG) signaling, both eCB-LTD in the nucleus accumbens (NAc) and reward-seeking behavior are restored in D1miRmGluR5 mice. In conclusion, we have identified a molecular and physiological mechanism within the NAc, in which cannabinoid and glutamatergic systems act in concert that is necessary and sufficient for driving reward-seeking responses. Our data suggest a common form of synaptic plasticity that underlies natural and drug reward seeking responses.

Association of NPY Receptor 2 Polymorphism with Alcohol Dependence

Presenting Author: Josef Frank

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Background: Neuropeptid Y (NPY) is an ubiquitous neurotransmitter and expressed in CNS and peripheral tissue. NPY is one of the most evolutionarily conserved peptides with 92% sequence identity between species with an evolutionary distance of more than 400 million years. NPY and it's receptors play an important role in the stress response system and are involved in the control of appetite and body weight homeostasis. These genes have been implicated in multiple diseases related to the stress response system (e.g. vascular diseases, obesity). In a similar manner these genes have repeatedly been reported to influence ethanol sensitivity and alcohol consumption. In a recent study on male fruit flies (drosophila melanogaster) the NPY homologue NPF has been suggested to be part on the brain's reward system and experimentally induced down regulation of NPF receptors has been shown to influence ethanol preference of the subjects under study. Interestingly candidate gene studies have found polymorphisms located in NPY and it's receptor genes associated with alcohol dependence (AD). We systematically explored single nucleotide polymorphisms (SNPs) located in these genes regarding their association with AD.
Methods: We systematically examined SNPs of the human NPY/NPY-receptor system in an already available GWAS data set comprising of 1333 inhouse patients with DSMIV-AD and 2168 population based controls.
Results: 24 markers of NPY and it's receptor genes were represented in the data set.SNP rs6857715 located in the promoter region of the NPY2R receptor gene was significantly associated with AD (p=4.7e-3).
Discussion: Findings are consistent with the literature. Interestingly we found the same SNP as Wetherill et al. (2009) had reported to be associated with comorbid alcohol and cocaine dependence.

Long-term ethanol effects on acute stress responses: Modulation by dynorphin

Presenting Author: Ildikó Rácz

Ildikó Rácz(1), Astrid Markert(1), Daniela Mauer(1), Birgit Stoffel-Wagner(2), Andreas Zimmer(1)

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The brain stress-response system is critically involved in the addiction process, stimulating drug consumption and the relapse to drug taking in abstinent addicts. At the same time, its functioning is affected by chronic drug exposure. Here, we have investigated the role of the endogenous opioid peptide dynorphin as a modulator of effects of long-term ethanol consumption on the brain stress-response system. Using the two-bottle choice paradigm we demonstrate an enhanced ethanol preference in male dynorphin knockout mice. Exposure to mild foot shock increased ethanol consumption in wild type control littermates, but not in dynorphin deficient animals. Blood ACTH levels determined 5 minutes after the shock were not affected by the genotype. We also determined the neuronal reactivity after foot shock exposure using c-Fos immunoreactivity in limbic structures. This was strongly influenced by both genotype and chronic ethanol consumption. Long-term alcohol exposure elevated the foot shock-induced c-Fos expression in the basolateral amygdala in wild type animals, but had the opposite effect in dynorphin deficient mice. An altered c-Fos reactivity was also found in the periventricular nucleus, the thalamus and the hippocampus of dynorphin knockouts. Together these data suggest that dynorphin plays an important role in the modulation of the brain stress-response systems after chronic ethanol exposure.

Genetic variation in the artrial natriuretic peptide transcription factor GATA4 modulates amygdala responsiveness to alcohol cues und relapse risk in alcohol-dependent patients

Presenting Author: Anne Richter

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Converging evidence from both preclinical and clinical studies suggests an involvement of atrial natriuretic peptide (ANP) in the symptomology of alcohol withdrawal and dependence. Moreover, two independent GWAS recently showed an association of single-nucleotide polymorphism (rs1327367) located in the gene encoding a transcription factor of ANP (GATA4) with alcohol dependence. This study examined whether amygdala reactivity to alcohol cues, a biomarker associated with addictive behaviour, differs depending on GATA4-genotype and whether these differential activations influence relapse behaviour.
81 alcohol-dependent patients completed an fMRI cue-reactivity task in a 3T scanner, during which they had to passively view alcohol-associated and neutral pictures. Follow-up data was available from 48 patients. Significantly lower alcohol cue-induced activations in the bilateral amygdala were found in G-allele carriers (ROI: $p<.05$ FWE-corrected; kE = 10), the group previously shown to be at a higher risk of relapse. Moreover, a subsequent survival analysis revealed that in AA-allele carriers higher amygdala activation significantly predicted a lower risk of relapsing to heavy drinking ($p = .018$). These results suggest a GATA4-genotype dependent protective effect of increased amygdala activation in response to alcohol relative to neutral stimuli. They further illuminate potential underlying mechanisms of the involvement of the GATA4 gene in the aetiology of alcohol dependence.

Convergent Functional Genomics in Alcohol Addiction Research - A Translational Approach to Identify and Study New Candidate Genes

Presenting Author: Jens Treutlein

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To identify susceptibility genes for alcohol dependence (AD), we conducted a genome-wide association (GWAS) and follow-up study. In a combined analysis, two linked intergenic SNPs on chr2q35 met genomewide significance. Long time ago, the 2q35 region has been reported to be implicated in a linkage study for a low level of response to alcohol. We enlarged our GWAS to 1333 patients and 2168 controls and conducted a pathway/geneset-based analysis by applying the global test analytical method to the pathway descriptions from BioCarta (dbBC), KEGG (dbKEGG), Reactome (dbRC), Gene Ontology (dbGO) and MSigDB (dbMIR, dbTFT, dbPOS). A group of associated pathways retrieved from the different sources highlighted the variants mapped to the gene X-ray repair complementing defective repair in Chinese hamster cells 5 (XRCC5), which is located on chr2q35. XRCC5 was one of the 156 top loci in a recently published gene set enrichment analysis of a GWAS for the level of response to alcohol. Association in the 2q35 chromosomal region was reported to be replicated independently in a GWAS for symptoms of AD. In mice, XRCC5 was reported as candidate gene for ethanol sensitivity, as it was differentially expressed in ´inbred short sleep´ compared to ´inbred long sleep´ strains, which differ in duration of loss of the righting response following a sedative dose of alcohol. As the gene and its function are conserved across a wide range of organisms, we next investigated its involvement in traits related to AD in a Drosophila model. The first line of experiments revealed that mutants of Drosophila Ku80, a homolog of mammalian XRCC5, were more resistent to the effects of ethanol on postural control. The effect of increased alcohol resistance to alcohol was present when Ku80 was impaired in neuronal cells in general, but not when restricted to dopaminergic neurons, indicating that Ku80 plays a role for alcohol responsivity in neuronal populations different from those of the dopaminergic system.

Genome wide association study of recently found new Alzheimer candidate genes in a German cohort combined with endophenotypes

Presenting Author: Wei Gu

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Introduction: Recent powerful GWAS identified markers close to the genes PICALM, CR1, CLU and BIN1 as novel risk loci for AD. Further multi stage meta analyses including our German cohort, identified additional markers close to the genes ABCA7, the MS4A cluster, CD2AP, CD33 and EPHA1. Despite strong association signals neither the exact location nor the biological, respectively clinical functions of these genes/genetic variants has been determined in patient samples so far. Failing to identify common coding variants in these genes leads to the hypothesis that numerous rare variants residing within or /close-by these loci are causative for the disease association. In order to elucidate the biological role of the novel risk-genes we examined possible associations with quantitative clinical measures intimately related to the disease.
Methods: This approach was based on 784 AD patients, genotyped using Illumina assays (550K and 610K). The clinical measures included detailed neuropsychological tests (CERAD-NP; MMSE, clock drawing), a variety of biochemical blood parameters and counts (e.g. VLDL, LDL, HDL, etc.), CSF parameters (Aß42 and Tau protein) and cranial MRIs. To increase the density of SNP marker for the risk-loci we performed an imputation using the 1000 genome datasets as reference. Associations are tested using linear regression with age, gender and duration of the disease (when appropriate) as covariates.
Results: We have identified several clinically interesting and functionally sound associations: e. g. for immune/inflammatory genes CR1 and CD33 with CSF Ab42 levels ($p<0.005$); for BIN1 and CD2AP playing a role in synaptic function with learning capabilities ($p<0.001$); for PICALM with resting serum levels of triglycerides ($p<0.002$).
In conclusion, the associations identified in this study corroborate an important involvement and function of the newly identified risk-genes on AD related endophenotypes and helped to narrow down the previous association signals.

Computational identification and experimental validation of microRNAs binding to the Alzheimer-related gene ADAM10

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Alzheimer's Disease (AD) is a progressive neurodegenerative disease implicating cognitive impairment and neuronal loss. One main event during AD-pathogenesis is the increased proteolytical cleavage of the Amyloid Precursor Protein (APP) by the beta-secretase BACE-1 (beta site cleaving enzyme 1). This results in generation of so-called A-beta peptides, which are the key components of AD-characteristic plaque-deposits in the brain of patients. Alternatively, APP can be cleaved by the alpha-secretase ADAM10 (a disintegrin and metalloproteinase 10) within the A-beta stretch. This prevents the formation of toxic A-beta peptides and moreover gives rise to the neuroprotective soluble fragment APPs-alpha.
Along AD-pathogenesis the expression of both, BACE-1 and ADAM10, is shifted in favour of BACE-1, thus generating pathological levels of A-beta peptides, which then contribute to disease progression. Besides regulation by transcription factors, one possibility to influence gene-expression is mediated by microRNAs (miRNAs), which generally suppress the expression of their target-genes. Therefore, identifying miRNAs which down-regulate the alpha-secretase ADAM10 could contribute to a deeper understanding of the molecular mechanisms underlying AD-pathology.
We established a bioinformatics-based screening approach to identify miRNAs, which potentially regulate the amount of ADAM10 (Augustin R. et al. BMC Med Genet, 2012). Results were evaluated experimentally by e.g. reporter-gene assay. Using this combined approach, we were able to characterize miRNAs, which probably regulate ADAM10 expression via translational regulation and therefore interfere with the pathomechanism of AD.

Several promoter CpGs are strongly associated with beta amyloid levels in post-mortem Alzheimer`s disease brain tissue

Presenting Author: Matthias Riemenschneider

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Alzheimer's disease (AD) is the most common form of dementia among the elderly. A key feature at the molecular level is the extracellular deposition of beta-amyloid peptides (Aß40 and Aß42) into senile plaques. The Aß peptides are generated by consecutive proteolytic cleavage of the APP protein via the beta-secretase (BACE1) and the gamma-secretase complex (PSEN1, PEN-2, Nicastrin and APH-1). During the last decades, large efforts have been made to decipher the genetic contribution to AD and it became evident that AD is a genetically complex, multi-factorial disease with environmental, genetic and epigenetic components involved.

To explore a possible contribution of DNA methylation to the pathogenesis of AD and key mechanisms intimately linked to AD (e.g. beta-amyloid levels), we performed a genome-wide methylation analysis using post-mortem temporal cortex samples (43 AD and 25 controls) on the HumanMethylation27k chip (Illumina), interrogating more than 27.000 CpGs distributed over ~14.000 gene promoters. In addition, soluble Aß40 and Aß42 concentrations were measured using protein extracts of the same samples by applying isoform specific ELISA's.

Using a multiple linear regression approach, we identified several CpG methylation sites showing strong association with Aß40 and Aß42 levels. Among these, we obtained a remarkable signal at the APH1B promoter (p=5.4x10-6, uncorrected). This is of particular importance, as the APH1B protein has been shown to contribute essentially to the total gamma-secretase activity and thus the generation of beta-amyloid peptides. In addition, our RT-PCR analyses demonstrate methylation dependent alterations of gene expression for a subset of the identified loci.

Our results suggest a considerable epigenetic contribution to the development of late-onset AD and AD assoc. pathological mechanisms. In particular, our findings demonstrate a so far unknown epigenetic mechanism, which modulates the gamma-secretase activity.

Genome-wide microRNA expression profiling in Alzheimer's disease

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MicroRNAs are small non-coding RNAs that are involved in different cellular and developmental processes by mediating post-transcriptional gene regulation. Dysregulation of their biological functions has already been associated with various disorders like cancer and cardiovascular diseases as well as immunological and psychiatric diseases. More recently several studies identified a potential involvement in neurodegenerative disorders like Parkinson, Huntington and Alzheimer's disease (AD), which is the most common form of dementia affecting millions of people worldwide. In this study, we examined expression patterns of >1200 miRNAs in human temporal cortex of 39 AD and 26 control samples using the Febit microarray system (miRBase16). Statistical analysis was performed using either Wilcoxon-Mann-Whitney-U or student's t-test subsequently adjusted by Benjamini-Hochberg resulting in two significantly down-regulated miRNAs that belong to miR-132 cluster (p[miR-132]=0.002; p[miR-212]=0.01). Deregulation of miR-132 and miR-212 has so far been associated with disorders like HD, schizophrenia, FTD, autism and PSP. Furthermore, there is evidence that miR-132 also influences inflammatory processes and cholinergic signaling by targeting acetylcholinesterase. To validate the results of this initial screening, we performed RT-PCR on a set of eight age and gender matched case/control samples from temporal (TP) and frontal cortex (FR) using appropriate TaqMan assays for the miRNAs of interest. In TP we could validate the results whereas in FR no significant deregulation has been observed between cases and control samples.

By using linear regression analysis we observed that the expression levels of miR-132 and miR-212 are also negatively correlated with increasing Braak stages (p[miR-132]=1.24*10-8; p[miR-212]=2.31*10-9).

Further experiments in AD mouse models in comparison to wildtyp mice are in progress to elucidate the link between miRNA expression and progression of the disease.

Generating a Reporter mouse which allows facilitated assessment of ADAM10 transcriptional activity in vivo

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Alzheimer‘s disease (AD) is the most common form of dementia. One of the elicitors is the formation of ß-amyloid-plaques, these occur as a result of the socalled amyloid-processing. Thereby, the amyloid-precursor-protein (APP) is processed by different secretases. One way is the cleavage through BACE1, which leads to the formation of sAPPß and through a further cleavage by a γ-secretase to Aß. On the other hand, ADAM10 cleaves within the amyloid-ß sequence of APP and though prevents the release of toxic Aß peptides. Therefore, ADAM10 is a potential target in therapeutic treatment of AD. One possibility is to enhance the gene expression of ADAM10, which has been shown to result in a reduction of plaque formation in transgenic mice. To identify possible substances which enhance the ADAM10 gene expression in vivo, we are generating an ADAM10-promoter-reporter mouse. Thereby the firefly luciferase cDNA is integrated in the endogenous ADAM10 gene-locus and is expressed under the control of the ADAM10 promoter. If the ADAM10 promoter is activated by a substance the expression of the luciferase is induced and can be measured as emitted light upon substrate administration.
The substances which should be tested in vivo are plant extracts which were identified as enhancers of the ADAM10 gene expression in cell culture.

The clinical spectrum of PRRT2 mutations - new mutations in PKD, ICCA and BFIS

Presenting Author: Felicitas. Becker

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Recent studies reported mutations in the gene encoding the proline-rich transmembrane protein 2 (PRRT2) to be causative for paroxysmal kinesigenic dyskinesia (PKD), PKD combined with infantile seizures (ICCA), and benign familial infantile seizures (BFIS). PRRT2 is a presynaptic protein which seems to play an important role for exocytosis and neurotransmitter release. Here, we sequenced PRRT2 in 14 sporadic and 8 familial PKD and ICCA cases of Caucasian origin and in 49 BFIS families and three sporadic cases of Italian, German, Turkish and Japanese origin. In the PKD/ICCA cohort, all ICCA families and 3 sporadic PKD cases showed previously detected mutations in PRRT2 (c.649dupC, c.291delC) Furthermore, we found three novel mutations (c.919C>T/p.Gln307*, c.388delG/p.Ala130Profs*46, c.884G>A/p.Arg295Gln) predicting two truncated proteins and one probably damaging point mutation. In the BFIS cohort, we identified the most common mutation c.649dupC in 39 of our families and one sporadic case (77% of index cases). Additional, three novel mutations were found in three other families, whereas 17% of our index cases did not show PRRT2 mutations, including a large family with late onset BFIS and febrile seizures. Our study reveals PRRT2 as the major gene for BFIS and PKD alone. In keeping with the published data, PRRT2 occur significantly lower in sporadic compared to familial cases. This suggests that other loci are relevant for sporadic forms of PKD, BFIS and ICCA, that other unknown, non-genetic factors play a role and/or that there are more diagnostic difficulties or uncertainties when only one affected family member exists.

Migraine without aura: genome-wide association analysis identifies several novel susceptibility loci

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Background. Migraine is a common primary headache disorder, characterized by attacks of severe, throbbing headache and autonomic nervous system dysfunction. In up to 30% of patients, attacks may be preceded by transient neurological disturbances (migraine with aura, MA), while the majority of patients have migraine without aura (MO). An important genetic component of migraine has long been recognized. However for the common, genetically complex types of the disease, hardly any solid data were available until recently. In 2010, the International Headache Genetics Consortium, by means of a genome-wide association study (GWAS), found a variant on chromosome 8q22 (close to MTDH) to be significantly associated with MA (Anttila et al., Nat. Genet. 2010). A population-based study subsequently identified three further risk variants for general migraine (Chasman et al., Nat. Genet. 2011). Based on these promising results, we here focused specifically on genetic susceptibility to MO, i.e. the most prevalent subtype.

Methods: In the discovery stage, data from two large clinic-based MO cohorts from Germany and the Netherlands (n = 2,326) as well as from 4,580 population-matched controls were subjected to genome-wide association analysis. The most promising signals (loci with = 2 SNPs with P-values < 1 x 10-5) were subsequently tested for replication in 2,508 Dutch, Spanish, Finnish and Norwegian patients and 2,652 controls.

Results: Meta-analysis of the discovery and replication data yielded four genome-wide significant MO susceptibility loci in or nearby MEF2D, PHACTR1, ASTN2 and TGFBR2. SNPs in two additional loci (in or near TRPM8 and LRP1), previously identified in a population-based GWAS of general migraine, were significantly replicated in our clinic-based MO cohort.

Conclusion: Our results establish the first susceptibility loci specifically for MO and open the door for a better understanding of the molecular pathophysiology of this debilitating headache disorde

Genomic analyses of Levetiracetam resistance in human epileptic tissue

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Focal epilepsies represent severe neurological disorders, which frequently originate in the temporal lobe (temporal lobe epilepsy; TLE). TLE is often associated with pharmacoresistance and in many TLE patients only neurosurgical removal of the seizure focus results in seizure control. Levetiracetam (LEV) represents a unique type of anti- epileptic drug (AED) as it is the only one known so far whose high-affinity binding site, the synaptic vesicle protein SV2A, is a component of the presynaptic release machinery. LEV generally leads to excellent seizure control even in previously refractory patients. However, a subgroup of LEV-treated TLE patients (approximately 20-30% of individuals) does not reveal any response to LEV from the beginning of treatment, i.e. a priori non-responders. This unexpected phenomenon is in contrast to the well known secondary acquired pharmacoresistance that is observed in a high number of patients. Using human hippocampal tissue derived from epilepsy surgery (n=52) we established a genome wide expression array analysis, which provides differential hippocampal gene expression patterns in LEV-responders versus a priori non-responders. Subsequent promoter analysis revealed individual single nucleotide polymorphisms (SNPs) that are strong candidates to influence the respective gene expression, such as of the molecule PIGP an elementary component of Wnt-signaling. Our results suggest distinct SNPs, transcription factors and presynapse associated molecules as new factors in LEV-response of TLE patients, which will need further assessment as diagnostic markers or therapeutic targets in the future.

Acknowledgement: Our work is supported by BMBF, NGFNplus (EmiNet).

Rs6295 promoter variants of the serotonin type 1A receptor correlate to respective mRNA expression in human epileptic brain tissue

Presenting Author: Katharina Pernhorst

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Many brain disorders, including epilepsy, migraine and depression, manifest with episodic symptoms that may last for various time intervals. Transient alterations of neuronal function such as related to serotonin homeostasis generally underlie this phenomenon. Several nucleotide polymorphisms (SNPs) associated with these diseases have been described. For obvious reasons, their functional consequences in human brain tissue remain largely enigmatic. Here, we have analyzed the potential of the SNP's rs6295 G-/C-allelic variants located in the promoter region of human 5HT1AR to modify expression of the respective gene coding for the G protein-coupled receptor 5HT1A (5HT1AR). The G-allele of rs6295 is known to be associated with aspects of major depression and migraine.

We have isolated DNA and mRNA from fresh frozen hippocampal tissue of pharmacoresistant temporal lobe epilepsy (TLE) patients (n=140) after epilepsy surgery for seizure control. We carried out SNP genotyping studies and mRNA analyses in order to determine 5HT1AR mRNA expression in human hippocampal samples stratified according to the rs6295 allelic variant. Strikingly, the mRNA expression of 5HT1AR was significantly more abundant in hippocampal mRNA of TLE patients homozygous for the rs6295 C-allele as compared to those with the GG-genotype. Our subsequent bioinformatic promoter analyses predicted a reduced binding affinity of the transcription factor (TF) c-Jun for the rs6295 G-allele. We tested this prediction by in vitro luciferase transfection assays. Increased levels of c-Jun resulted in a significantly stronger activation of the rs6295 C-allelic variant in comparison to the rs6295 G-allele. These data may point to a novel rs6295 allelic variant and c-Jun dependent transcriptional 5HT1AR 'receptoropathy'.

Functional analysis of novel SCN2A mutations found in patients with infantile epilepsies.

Presenting Author: Mariana Zaichuk

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Benign familial neonatal-infantile seizures (BFNIS) is an epileptic syndrome, characterized by afebrile generalized seizures with the mean seizure onset of 11 weeks and spontaneous remission within the first year of life. A number of mutations in the gene encoding the NaV1.2 sodium channel (SCN2A) were linked to this disease. NaV1.2 is a neuronal voltage-gated sodium channel jointly responsible for the generation and propagation of action potentials.

The aim of this study is to characterize the functional consequences of three novel NaV1.2 mutations. The first mutation, Y1589C, was identified in a family with benign infantile seizures, originating from Madagascar. The family consists of 9 affected individuals in 3 generations. The V208E missense mutation was found in a severely affected female patient in Switzerland. Other, less severely affected family members also carry the mutation. The third mutation, R28C, was found in a benign case of BFNIS.

To characterize the effects of these mutations on the channel function we co-transfected tsA 201 cells with wild-type or mutant NaV1.2 a subunits together with both ß1 and ß2 subunits. We used the whole cell patch clamp technique to record Na+ currents.

All three mutations caused gain-of-function effects compared to the WT channel. The Y1589C mutation induced a depolarizing shift of steady-state inactivation and increased the inward Na+ current at subthreshold voltages as recorded using action potential waveforms as voltage clamp protocols derived from pyramidal neurons in brain slices. The V208E mutation resulted in a shift of voltage-dependent activation towards more negative membrane potentials. Mutation R28C was leading to a similar increased subthreshold Na+ current as Y1589C. These findings can explain the origin of a hyperexcitability of pyramidal/principle neurons which highly express NaV1.2 channels during early postnatal development which is a plausible explanation for the generation of infantile seizures.

Integrating protein-protein-interaction networks with experiment-based quality scores and context information for the detection of reliable and meaningful interactions

Presenting Author: Martin H. Schaefer

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The detection of protein-protein-interactions (PPIs) and the analysis of the networks they form is of increasing importance for the understanding of biological mechanisms and their perturbation in disease. Informative network models should be reliable and take cell type-specific characteristics into account. Reaching these goals is difficult given the partly error-prone experimental methods used to measure interactions between proteins and the often artificial or non-representative conditions under which the PPI screens are done.
To address these problems, we developed the resource HIPPIE (Human Integrated Protein Protein Interaction rEference; available at http://cbdm.mdc-berlin.de/tools/hippie), which provides human, experimentally detected PPIs and associated confidence scores. Its scoring scheme integrates experimental evidence tracks from multiple experimental PPI resources and has been optimized both computationally and by human experts to reflect the amount and quality of evidence for a given PPI. To take into account that PPIs are highly context-specific and dynamic, we integrated gene expression information and functional descriptions of proteins. This allows the construction of tissue-specific subnetworks or the extraction of PPIs dependent on other conditions. We used graph algorithms to infer network flow in context-specific networks to detect functionally important or disease-relevant interactions. We demonstrated that the combination of context information with network algorithms is able to select interactions of high biological relevance and can reproduce known pathways and canonical disease proteins. We applied this method to lung-specific networks and highlighted interactions potentially mediating the cross-talk between influenza virus proteins and cellular immune response.

Novel Variants in autosomal dominant Parkinson's disease: Exome Sequencing and Linkage Analysis

Presenting Author: Claudia Schulte

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The objective of this study was to identify putatively pathogenic novel mutations that cause ausomal dominant inherited forms of Parkinson's disease (PD).
The mapping and cloning of genes causing monogenic forms of PD have probably contributed most to the understanding of the molecular pathogenesis of PD in the last 10 years. Known mutations in two genes, SNCA and LRRK2, account for only 10 to 15% of PD with autosomal-dominant inheritance and 1 to 5% of apparently sporadic cases in most European populations. Nevertheless, it must be assumed that several other dominant loci await discovery.
A cohort of 15 PD patients from autosomal dominant PD families was exome sequenced. Additionally, 12 of these patients and their relatives were investigated in a linkage analysis. The PD families had at least two affected family members available, excluded for known dominant genes. Exome sequencing was performed using the SOLiD platform and sure select enrichment. Linkage analysis was carried out with 250k DNA microarrays (Affymetrix). Genetic variants identified by exome sequencing were genotyped in additional family members with Sanger Sequencing to validate the variant and assess segregation.
Analyses of data revealed several possibly pathogenic variants segregating in family pedigrees.
Further evaluation of novel variants in large PD cohorts will hopefully strengthen our results. The identification of novel genes will provide insights in the molecular pathways underlying the disease. This will hopefully lead to the identification of new targets for therapeutic interventions and might be useful for diagnostic applications.

The UBX protein ASPL rapidly converts functional p97 hexamers into non-functional heterotetramers

Presenting Author: Erich Wanker

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p97/VCP is a multifunctional ATPase that interacts with various adaptor proteins and influences their cellular function in a spatio-temporal manner. However, the number, size and biological properties of p97/VCP containing protein complexes in mammalian cells are currently unknown. Recently, we have identified ten novel p97/VCP associated proteins by automated Y2H interaction screening; one of these - the UBX containing protein ASPL - was characterized in detail. We found that ASPL directly associates with VCP hexamers and rapidly converts these structures into highly stable VCP:ASPL heterotetramers. The crystal structure of p97/VCP bound to a C-terminal fragment of ASPL revealed that a unique helical lariat structure extending the UBX domain is crucial for the disruption of inter-protomer interactions and the disassembly of p97/VCP hexamers. Site-directed mutagenesis of residues potentially facilitating the VCP:ASPL interaction abrogated the disassociation of hexamers. Overexpression of wild-type ASPL in mammalian cells induced the activation of effector caspases, which was not observed with a mutant ASPL variant that does not disassemble p97/VCP hexamers in vitro. Thus, our investigations provide biochemical and structural evidence that ASPL functions as a highly specific hexamer segregase, controlling the cellular activity of the AAA ATPase p97/VCP.

Impact of calpain cleavage of alpha-synuclein on the pathogenesis of Parkinson's disease in vivo

Presenting Author: Meike Diepenbroek

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Parkinson´s disease (PD) is a common neurodegenerative and slowly progressive disorder. Mutations and gene multiplications of the alpha-synuclein (aSYN) gene are known to cause familial PD. aSYN is a small soluble protein and it is expressed primarily at presynaptic terminals throughout the brain. The precise function of aSYN is unknow so far.
Calpain is a calcium-activated protease, and aSYN is a substrate for calpain cleavage. In the last years, it was shown that calpain plays a role in the aggregation of C-terminal truncated aSYN and contributes to the toxicity of fibrillized aSYN by cleaving within the C-Terminus. Therefore, our study is focused on the functional implication of calpain-mediated proteolytic cleavage of aSYN in the pathogenesis of PD.
In order to analyze the neurotoxic impact of calpain-cleaved aSYN we crossbred and characterized two complementaring mouse models, expressing human mutated [A30P]alpha-synuclein ([A30P]aSYN) either in mice overexpressing human calpastatin (cast-tg) or on a calpastatin-deficient background (cast(-/-)). Analysis of double-transgenic (dtg) and double-mutant (dmut) mice will reveal, whether inhibition or increased activation of calpain triggers the alpha synuclein-induced phenotype in these PD mouse models.
So far we were able to show that calpain 1 is the major calpain isoform that plays a role in the degradation of alpha-synuclein and that calpain is activated by knocking out the endogenous calpain inhibitor calpastatin in vivo.

Gain- and Loss-of LRRK2 – Comprehensive analysis of two Mouse Models

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Parkinson's disease (PD), caused by the progressive loss of dopaminergic neurons in the substantia nigra, is not only characterized by motor problems like tremor, rigidity or postural instability. But also pre-motor symptoms like mood disorders or olfaction deficits are known. The gene encoding the Leucine-Rich Repeat Kinase 2 (LRRK2) has been shown to be associated with late-onset autosomal dominant form of PD with pleomorphic pathology.
For further exploring the aetiology of PD, the c>t transition has been inserted into the GTPase domain of the endogenous murine Lrrk2 gene, mimicking the pathological mutation R1441C observed in Parkinson's disease patients. Furthermore, an existing knockdown mouse line has been utilized to analyze the effects of an almost complete loss of LRRK2 protein in vivo. The two mouse lines were comprehensively analyzed at different ages in regard to behavioural, morphological and pathological changes. Both lines did exhibit neither any overt neurodegeneration nor pathological hallmarks of PD like inclusion bodies. Also on the postsynaptic side of the affected nigrostriatal dopaminergic neurons, the detailed morphological analysis of the medium spiny neurons (MSN) in the adult striatum of both mouse lines could not reveal significant alterations. Nevertheless, comparing the results of the two lines on the behavioural level, both lines performed in a similar manner showing a high overlap in regard to the identified phenotypes (e.g. olfactory deficits and gait alterations). These results suggest that on the systemic level the postulated opposing molecular effects of pathogenic mutation (gain) versus a massive reduction of the protein (loss-of) are ending up in similar behavioural alterations.
Furthermore, based on the missing neurodegeneration, it seems that distinct mechanisms compensate the malfunction or loss-of function of Lrrk2 quickly. Hence these mouse lines can be utilized to identify and study putative compensatory mechanisms.

Association of rare variants in TCF4 gene with schizophrenia

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Schizophrenia is a severe psychiatric disorder with a complex genetic architecture. Up to date a number of common variants with small to moderate effect sizes and rare variants such as copy number variations with larger effect sizes are reported to contribute to the susceptibility of schizophrenia. One of the most robust findings arising from genome-wide association studies is the association of common genetic variation at the TCF4 locus with schizophrenia. The aim of this study was to investigate the potential contribution of rare variants such as single base substitutions and/or small insertions/deletions in the TCF4 gene to schizophrenia. We resequenced the protein coding exons and 50 base pairs up- and down-stream flanking sequences of the TCF4 gene in 191 schizophrenia patients and analyzed the data for low-frequency variants with a minor allele frequency (MAF) = 3%. We also screened the variants identified in the 379 European individuals in the same analysis frame from 1000 Genomes Project and followed-up all the variants (n=16) in a large sample of ~1800 schizophrenia patients and ~2250 control individuals. The data was analyzed by different statistical methods developed for association analysis of rare variants and a statistically significant overrepresentation of rare variants (MAF = 1%) was observed in patients. Our study strongly suggests that a spectrum of common and rare variants at the TCF4 locus contribute to the development of schizophrenia.

Duplications in RB1CC1 are associated with schizophrenia in large samples from Europe

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Schizophrenia is a severe and debilitating neuropsychiatric disorder with an estimated heritability of ~80%. Previous studies have shown that rare, highly penetrant copy number variants (CNVs) account for a fraction of the overall genetic risk. The disease-associated CNVs tend to span several genes and it is difficult to identify the disease underlying gene. Recently, single schizophrenia susceptibility genes have been identified using exome sequencing. We wondered whether those genes that carried de novo mutations in patients might carry additional risk variants, such as other rare single nucleotide mutations but also rare CNVs.

We tested this hypothesis by starting from 55 potential schizophrenia susceptibility genes that were reported to carry de novo mutations by Xu et al. (2011) and Girard et al. (2011). We analyzed whether CNVs at these loci might contribute to the allelic spectrum in schizophrenia. In our discovery sample of 1 637 patients and 1 627 controls duplications in RB1CC1 were more frequent in our patients than in the controls. This finding was followed-up in large, independent samples. In the combined analysis, totaling 7 750 patients and 112 007 controls, duplications in RB1CC1 were associated with schizophrenia (P-value = 0.016; odds ratio = 4.65).
Our study provides evidence for rare duplications in RB1CC1 being a risk factor for schizophrenia.

Clustering Analysis of Low-Frequency Risk Variants in the Schizophrenia-associated 1q21.1 Microdeletion Region Suggests CHD1L as Disease-Relevant Gene

Presenting Author: Andreas J. Forstner

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In large genome-wide surveys we and others have recently identified rare microdeletions on chromosome 1q21.1 as strong genetic risk factors for schizophrenia (odds ratio ~ 10). By systematic resequencing and follow-up genotyping we wanted to address the question whether the microdeletion region harbors other rare genetic risk variants and whether such variants occur preferentially in one or more genes within the deletion region. This may provide valuable information regarding the causative risk gene(s).

We performed exon-targeted Sanger sequencing of seven RefSeq genes in 94 DSM-IV-diagnosed schizophrenia patients and 94 controls. The identified variants with a minor allele frequency (MAF) of =5% were subjected to several statistical tests developed to analyze rare variants. Based on the results variants were followed up by genotyping in a large sample of ~ 1,900 schizophrenia patients and ~ 2,200 controls.

Altogether 55 variants with a MAF=5% were detected by resequencing. Clustering analysis revealed a significant risk association signal in the region for variants with a MAF=3% (p=0.028) and a MAF=5% (p=0.048). When the analysis was restricted to single genes, CHD1L was the only gene which showed a significant association (p=0.034, MAF = 3% and MAF = 5%). All variants in this gene were followed up in a larger cohort by genotyping. Results of the follow-up analyses will be presented.

Testing for spatial distribution of low-frequency variants is based on the biologically plausible hypothesis that different variants with the same effect cluster in the disease-relevant genomic region. Our results provide evidence for a significant clustering of low-frequency risk variants in schizophrenia patients in the 1q21.1 microdeletion region. A detailed analysis of single genes suggests that CHD1L may be the causative risk gene. These results may indicate new pathways in disease etiology and help us understand how the 1q21.1 microdeletion increases risk to develop schizophrenia.

Analysis of copy number variants in genes reported to carry a genome-wide significant SNP

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Based on family and twin studies it is well known that genetic factors are an important contributor to the susceptibility to neuropsychiatric disorders such as schizophrenia (SCZ), bipolar disorder (BPD), and major depressive disorder (MDD).

In the search of causal genetic variants several large genome-wide association studies (GWAS) have been performed and a small number of genome-wide significant SNPs have been identified. Despite these accomplishments, the search for the disease causal genes has been rather unsatisfactory and the identified SNPs can explain only a small proportion of the heritability. Recently, rare copy number variants (CNVs) have been identified as substantial risk factors for the development of SCZ and possibly BPD. The role of CNVs in MDD is still under investigation.

To our knowledge, is has not been studied so far whether CNVs affecting the genes the genome-wide significant SNPs have been allocated to might be enriched in patients with SCZ, BPD, or MDD compared to controls. Evidence from this CNV candidate gene study might provide additional support for an involvement of some of the identified candidate genes in SCZ, BPD, or MDD.

In the present study, we applied a candidate gene approach allowing us to search for smaller CNVs in our dataset. We screened 575 patients with MDD, 882 patients with BPD, 1,637 patients with SCZ, and 1,643 controls for the presence of CNVs in the candidate genes identified in GWAS. All individuals were genotyped on HumanHap550v3, Human610-Quadv1, and Human660W-Quad arrays (Illumina, CA, USA). The analysis is still ongoing and the results will be presented at the conference.

Pathway analysis of two GWAS cohorts for unipolar disorder

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Studying the cumulative effect of single nucleotide polymorphisms (SNP) assigned to functional gene sets, such as biological pathways, may help to discover and explain the mechanisms of risk SNPs on common diseases. As a complementary approach to the more often used single-locus case-control comparison approaches, the pathway analysis may allow to detect risk SNPs with small effects by reducing the complexity of the genetic tests through including biological and functional pathway and network information. The analysis of SNPs in groups predefined by biological knowledge as the unit of analysis can increase the power to detect association between genes and disease.

In order to carry out the pathway-based analysis, we used a pre-existing algorithm, Globaltest. The Globaltest was originally developed for gene expression data and can be applied to categorical variables. We assigned SNPs to a gene if the SNP was located within the genomic sequence and within 20 kb of the 5' and 3' ends of the first and last exons. We allowed a window of 20 kb on either side of the gene to account for important regulatory regions. If an SNP lies within a shared region of more than one gene, it was assigned to all genes. We applied the globaltest to SNP data coding the SNP effect as allele dosis effect (0, 1, 2). The functional gene sets information was retrieved from several pathway databases such as KEGG, Gene Ontology, and MSigDB. We validated the results in a two-stage (discovery/replication) pathway analysis of GWAS of unipolar disorder data set. The discovery set consisted of Bonn-Mannheim (BOMA) data sets and comprised 2172 affected and 1510 controls. The replication set consisted of Genetic Association Information Network (GAIN) data sets and comprised 1741 cases and 1761 controls in European Ancestry individuals. Results of this analysis will be presented.

Studies in humans and mice implicate neurocan in the etiology of mania

Presenting Author: Sandra Meier

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Objective: A recent study reported genome-wide association between the NCAN gene and bipolar disorder. The aims of the present study were to characterize the clinical symptomatology most strongly influenced by NCAN, and to explore the behavioral phenotype of Ncan knock-out (Ncan-/-) mice.
Methods: Genotype/phenotype correlations were investigated in patients with bipolar disorder (n=641) and the genetically related disorders major depression (n=597) and schizophrenia (n= 480). In a first step, principal component and genotype association analyses were used to derive main clinical factors from 69 lifetime symptoms, and to determine which of these factors were associated with the NCAN risk allele. In a second step, these analyses were repeated using the associated factor(s) only in order to identify the more specific clinical subdimensions that drive the association. Ncan-/- mice were tested using diverse paradigms assessing a range of behavioral traits, including paradigms corresponding to bipolar symptoms in humans.
Results: In the combined patient sample, the NCAN risk allele was significantly associated with the mania factor, in particular the subdimension overactivity. Ncan-/- mice were hyperactive, and showed more frequent risk-taking and repetitive behaviors, less depression-like conduct, impaired pre-pulse inhibition, amphetamine hypersensitivity, and increased saccharin preference. These aberrant behavioral responses were normalized following the administration of lithium.
Conclusions: NCAN preferentially impacted on mania symptoms in humans. Ncan-/- mice showed behavioral abnormalities that were strikingly similar to those of the human mania phenotype, and may thus serve as a valid mouse model.

Genome-wide association study reveals four new risk loci for bipolar disorder

Presenting Author: Thomas W. Mühleisen

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Bipolar disorder (BD) is a severe disorder of mood, characterized by recurrent episodes of mania and depression. The genetic factors underlying the etiology of BD are still largely unknown, although the estimates of heritability for BD are high and range between 60% and 80%. Since the first genome-wide association study (GWAS) of BD in 2007, a handful of risk loci at the widely acknowledged formal threshold of genome-wide significance (P<5E-08) could be identified which replicated in adequately sized follow-up studies, notably ANK3, NCAN, CACNA1C, and ODZ4. The first genetic findings for BD together explain only a small proportion of the heritability but experimental data strongly suggests that a substantial portion of the heritability may be explained by hundreds of different risk loci of very small genetic effect. One crucial step towards the identification of additional loci should be amenable by increasing the sample sizes.

In the BMBF-funded MooDS consortium, we generated a second wave of GWAS data from 2,266 BD patients and compared them with 5,488 controls originating from 6 European countries and Australia who have not been investigated in a discovery step for BD before. To further increase the statistical power, we combined our MooDS data with published data from the Psychiatric GWAS consortium, resulting in the currently largest GWAS sample studied in BD (9,747 patients, 14,738 controls). Our analysis in 2.4 million SNPs revealed 47 SNPs reaching genome-wide significance at 7 loci, four of which were novel (2q11.2, 5p15.11, 6q16.1, and 7p21.3). Our findings suggest a role of neurodevelopmental processes, and - for the first time through GWAS - signal transduction at G-protein coupled receptor pathways in the etiology of BD. Our study demonstrates that an increase of GWAS sample size will likely pinpoint many more genetic factors in BD and gradually increase our knowledge about the biological processes involved in this common neuropsychiatric disorder.

Alopecia Areata: Genetic and Psychological Factors

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Introduction: Alopecia Areata (AA) is an autoimmune, inflammatory disease directed against the hair folicle with a life-time prevalence of 1.7%. It is characterized by patchy loss of the scalp hair and/or body hair. Genetic factors are contributing: the life-time prevalence of first degree relatives of AA patients is 3 to 4 times increased. The increased co-morbidity between AA and Major Depression (MD) in small studies suggests interplay of psychological factors on AA and MD risk.
Aim: To investigate whether MD rate is increased in AA patients of a large case-control samplen and associated with AA severity; AA patients with a positive family history of psychiatric disorders (FH psych+) have an increased MD rate; the association between age of onset (AaO) of AA and AaO of MD is indicative that one of the two disorders occurs first; and AA patients without MD have a higher genetic load for psychiatric disorders (i. e. FH psych+) than controls without MD which would be indicative of a common ethological link of AA and MD.
Methods: 607 patients and 1202 controls responded to a questionnaire assessing life-time MD and FH psych+ in first degree relatives.
Results: 32% of male and 47% of female AA patients met criteria of lifetime MD as compared to 15% and 24% of controls. In women, MD rate was depending on AA severity. The MD rate in AA patients with FH psych+ was significantly increased. There was no statistical indication that the one of the two disorders occurs first. Male AA patients with MD reported a higher FH psych+ rate than male controls with MD.
Conclusion: In AA patients, MD rate is 2.5-fold increased compared to controls. AA severity influences MD rate in women. All patients with AA should routinely be screened for depressive symptoms and if necessary receive professional treatment of their depression. Based on the findings, we hypothesize that the increased co-morbidity between MD and AA is due to a mutual dependency.

Transcription profiling and pathway analysis in euthymic and manic bipolar patients and controls

Presenting Author: Stephanie H Witt

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We conducted a blood-based microarray investigation using Human Exon 1.0 ST Arrays (Affymetrix) in a sample of inpatients with a diagnosis of BD in their euthymic and manic phase (N=10). Diagnostic groups were compared to controls, and euthymic and manic phases were compared with each other. Analyses of covariance comparing mean expression levels on a gene-by-gene basis were conducted to generate the top significantly dysregulated gene lists for patients by each diagnostic group and controls. A pathway-based analysis was conducted by applying the global test analytical method to KEGG pathways.
Results show over 200 differentially expressed genes comparing patients in the manic or euthymic phase with controls respectively. A subset of the significant hits were differentially expressed comparing the manic and euthymic phases and in both manic and euthymic phases vs. controls, and there was an overlap of genes which showed differences in expression comparing euthymic phase vs. controls and euthymic vs. manic phase. Pathway analyses demonstrated distinct clusters of enriched pathways in the BD manic vs. control comparison group.
Findings provide evidence of general and specific genetic regulation in manic and euthymic phases of BD. Although the results are preliminary, they suggest that significantly more genes are differentially regulated in the manic and in euthymic phase compared to controls than in manic compared to euthymic phase with a partial but not complete overlap between differentially expressed genes in the two bipolar phases. The results of this approach could lead to the identification of new genes and molecular and cellular pathways that contribute to the development of BD and thus support further investigation of for the identification of biomarkers for BD.

Whole-exome sequencing in extended families with myocardial infarction

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Genome-wide association studies (GWAS) have in the last years identified several chromosomal loci for coronary artery disease (CAD) and myocardial infarction (MI). However, only a small fraction of the expected heritability is explained so far. A drawback of GWAS is the lack of power to detect rare yet functionally important variants.
Here we analyze two extended families, out of a collection of 22 extended MI- families, with clustering of MI/CAD by means of whole-exome sequencing (WES) to reveal rare disease-causing variants. In both families, we selected three distantly related affected family members for sequencing. The three affected family members, in each family, share approximately 20,000 variants. We expect the disease-causing variant to be rare, hence we first filtered based on a frequency >1% in dbSNP. Next, we removed variants in non-conserved regions and in regions of segmental duplication. Finally, we filtered the remaining variants based on predicted function using Annovars Avsift and SNPEFF.
Due to the relative large number of potential causal variants left in both families, we first focused on those not found in dbSNP (132). This approach left two and three potential variants in the two families, respectively. After Sanger re-sequencing, one remained in each family; a splice-site mutation in the LDLR gene (IVS9-1G>A) and a base-pair substitution that cause a stop-codon (g.120548312T>A) in the PDE5A gene. The PDE5A-variant lies in a strong regulatory region and may hence also influence transcription. Both variants show co-segregation with the disease.
Here we show that WES is a powerful tool to identify the disease-causing variant within families. Moreover, this study demonstrate the first step towards personalized medicine. The identification of the disease-causing variant in the LDLR gene by WES enables a preventive treatment by lowering LDL cholesterol of mutation carriers not having developed the disease so far.

Coronary artery disease risk loci identified in over 190,000 individuals implicate lipid metabolism and inflammation as key causal pathways

Presenting Author: Christina Willenborg

The CARDIoGRAMplusC4D Consortium

Universität zu Lübeck

Coronary artery disease (CAD) is the commonest cause of death. Here we report an association analysis in 63,746 CAD cases and 130,681 controls identifying 15 loci reaching genome-wide significance, taking the number of such loci for CAD to 46, and a further 104 independent variants (r2<0.2) strongly associated with CAD 5% FDR). Together these variants explain approximately 10.6% of CAD heritability. Of the 46 genome-wide significant lead SNPs, 12 demonstrate a significant association with a lipid trait and 5 with blood pressure but none with diabetes. Network analysis with 233 candidate genes (loci at 10% FDR) generated five interaction networks comprising 85% of the putative CAD genes. The four most significant pathways mapping to these networks are linked to lipid metabolism and inflammation underscoring their causal role in the genetic aetiology of CAD. Our study provides novel insights into the genetic basis of CAD and identifies key biological pathways.

Gene expression program during cardiac growth in SHRSP shows variability associated with hypertensive left ventricular hypertrophy

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Research question: Reactivation of fetal gene expression patterns has been demonstrated to play a crucial role in common cardiac diseases in adult life including left ventricular hypertrophy (LVH). Thus, increased wall stress and neurohumoral activation are discussed to induce the return to expression of fetal genes after birth in LVH. We therefore aimed to test whether fetal gene expression programs are linked to the genetic predisposition to LVH. We performed genome-wide gene expression analysis in a genetic rat model of LVH, i.e. the stroke-prone spontaneously hypertensive rat (SHRSP), to identify differences in expression patterns between day 20 of development (E20) and week 14.

Methods: Genome-wide gene expression analysis was performed by microarray-technology. We extracted RNA from heart tissue of F344 and SHRSP rats (n=6, respectively) at E20 and week 14. We compared gene expression at both time points in each strain to identify different expression patterns during development between F344 and SHRSP. Statistical analysis was performed using packages "lumi" and "limma" from Bioconductor for R programming environment.

Results: We identified overall more than 100 genes with differential expression between E20 and week 14 in each strain, respectively. 50 genes were differentially expressed only in SHRSP and 45 only in F344, respectively. Further bioinformatic analysis presented Card9 (caspase recruitment domain family, member 9) and Efcab6 (EF-hand calcium binding domain 6) as potential candidate genes showing similar expression rate in E20 in both strains but significantly upregulation only in adult SHRSP rats.

Conclusion: Our analysis of gene expression program during development in rats with genetic hypertension identified Card9 and Efcab6 as new candidate genes for LVH. They represent thus interesting novel targets for further functional analyses and the elucidation of mechanisms leading to LVH.

Advanced GWAS interpretation in R

Presenting Author: Frank Rühle

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We present 'postgwas' and 'boostSeq', two comprehensive toolkits for post-processing, visualization and advanced analysis of results from genome-wide association studies (GWAS). We attempt to unify and simplify several standard procedures that are essential for the interpretation of GWAS including generation of Manhattan and regional plots. We have implemented novel interaction network analysis tools for the investigation of systems-biology aspects and functionality of rare variant analysis as well as algorithms for selection of the most informative samples for subsequent next generation sequencing applications. The package supports virtually all model organisms and represents the first cohesive implementation of such tools for the popular programming language R. Unlike most comparable previous software it is adaptable for custom work pipelines. Here we present handling of to-date problems such as subphenotype comparison, pleiotropy detection and rare variant analysis as well as subsample selection for sequencing. The software packages can be downloaded from CRAN (http://cran.r-project.org/) and are freely available to the scientific community.

Dissecting the Role of microRNAs as Biomarkers for Cardiovascular Diseases

Presenting Author: Britta Vogel

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To elucidate the role of microRNAs as potential biomarkers for cardiovascular disorders, we performed genome-wide miRNA expression measurements in patients with diverse cardiovascular diseases. By applying advanced algorithms such as machine learning techniques, we were able to retrieve the diagnostic information within these complex miRNA profiles and show their potential as novel biomarkers.

As an example, we present data on microRNA expression patterns in patients with non-ischemic heart failure with reduced ejection fraction (HF-REF). We find several microRNAs that show altered expression levels in HF-REF patients, with miR-520d-5p being the most significantly dysregulated. Interestingly, we find a set of miRNAs directly correlated with systolic cardiac function measured by the left ventricular ejection fraction. With the help of a microRNA signature we were able to distinguish HF-REF patients from healthy individuals with high statistical power (AUC = 0.81).

In summary, our results highlight the potential of microRNAs as novel biomarkers for cardiovascular diseases und underline the importance of advanced bioinformatics methods in biomarker discovery studies.

Homology model of Abcc6 provides insight into the function of mutations causing cardiovascular phenotype

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Rare mutations in the ABCC6 gene have been demonstrated to cause pseudoxanthoma elasticum in humans as well as dystrophic cardiovascular calcification (DCC) in mice. A case-control study indicates that frequent DNA variants in the ABCC6 gene are not rare in the general population and contribute to an increased propensity toward premature atherosclerotic vascular disease. The exact biological function of ABCC6 as well as the functional relationship of this transmembrane protein to the pathogenesis of atherosclerosis or calcification are presently still unknown.

In this study we aim to get insights into the molecular effect of the Aa substitutions on protein function by molecular and computational methods. NZB mice are predisposed to DCC, whereas C57BL/6 mice strain remains resistant. Two base pair exchanges leading to Aa substitutions were found in NZB strain (A706V, I927T) and lead to a stable protein. Hence it follows that the mutations in Abcc6 in NZB mice may affect the protein function.

For investigation of the effect of this mutations on protein function we built three homology models (wtABCC6: M1, A706V-ABCC6: M2 and H1401Q-ABCC6: M3) and used these as starting point for our dynamic simulations.

Analyzing the position of A706V, the mutant is located near the domain-domain contact. Fülöp et al. suggested the importance of this region in context of functionality. So it is interesting that our dynamic simulation results, of M2 compared to M1, show a movement in this region.

H1401Q is located within the ATP-interface region. We observed a change in the strong conserved ATP hydrogen bonding network during the simulation of M1 compared to M3.

These two observations suggest that the mutations influence the domain-domain interaction and respectively the ATP-transport and consequently the protein function.

The experimental and computational results are consistent. In vitro experiments are ongoing to further confirm the importance of the two mutations.

Common variation in mitochondrial DNA is not associated with obesity

Presenting Author: Nadja Knoll

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Heritability estimates of the variance of the body mass index (BMI) are high. For mothers and their offspring higher BMI correlations have been described than for fathers. Variation(s) in the exclusively maternally inherited mitochondrial DNA (mtDNA) might contribute to this parental effect.

A maximum of 40 mitochondrial SNPs were available from genome-wide association study (GWAS) SNP (single nucleotide polymorphism) arrays (Affymetrix 6.0). In the discovery step, we analyzed association in a case-control sample of 1157 extremely obese children and adolescents and 434 lean adult controls. In the independent confirmation step, 7007 population-based adults (KORA, SHIP and POPGEN) were analyzed as case-control samples (BMI = 30 kg/m2 categorized as obese cases and BMI < 25 kg/m2 as normal weight or lean controls). SNPs were analyzed as single SNPs and as haplogroups (HaploGrep). Fisher's two-sided exact test was used for association testing.

In the discovery step, nominally significant association to obesity was found for the frequent allele of rs28358887 (p=0.0020), haplogroup M (p=0.0381) and haplogroup W (p=0.0194). rs28358887 is located in the ATP6 gene. These findings could not be confirmed in the population-based adults.

Our hypotheses of a contribution of mtDNA to the observed higher BMI correlations between mothers and offspring cannot be substantiated by the findings of the present study.

Metabolic Phenotyping of the Mouse Mutant Line Ftotm1b

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Single Nucleotide Polymorphisms in the FTO gene are associated with polygenetic obesity in human populations. The FTO protein is ubiquitously expressed. So far its molecular function and role in the development of obesity remains unclear. Fto knock out mice are smaller and develop a lean phenotype due to growth retardation after birth. In addition energy expenditure per gram body mass is elevated in these mice. In contrast to this first report a second Fto deficient mouse mutant line (MML) exhibits an obese phenotype.
We started to characterise a new Fto deficient MML. In this MML the third exon of the Fto gene was deleted resulting in a frame shift in the ORF. We observed a high postnatal lethality of pups homozygous for the Fto deficiency. Homozygous pups that survived until d21 had a lower body mass and body temperature than wildtype or heterozygous littermates. We confirmed a growth retardation phenotype in mice lacking Fto. Adult Fto deficient mice were smaller but had a higher fat mass and lesser lean mass than predicted for their body mass. Energy expenditure was increased when adjusted for lean mass. Food consumption and total activity were slightly decreased. In addition mice lacking Fto exhibited a clear behavioural phenotype with reduced levels of rearing behaviour. In conclusion the observed metabolic phenotype of a new Fto deficient MML suggests that loss of FTO function promotes obesity.

1000G imputations in a family-based genome-wide association study for genomic imprinting of early onset extreme obesity

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Introduction: Genome-wide association studies (GWAS) have had a dramatic impact on our understanding of obesity (Speliotes et al., 2010; Bradfield et al., 2012). Typically, GWAS focus on allelic effects of single "single-nucleotide polymorphisms" (SNPs). Thus, cases in whom an allele inherited from one parent is expressed at a significantly lower level than the same allele from the other parent are largely ignored. Family-based GWAS provide the option to assess this epigenetic process of genomic imprinting.

Material and Methods: We extended our previous work using the 1000 Genomes data set for imputations (http://www.1000genomes.org). We performed a genome-wide imprinting analysis of 705 German nuclear families with extremely obese offspring originally genotyped by the Affymetrix Genome-Wide Human SNP Array 6.0 data. We analysed the imputed data set by stratified transmission-disequilibrium-tests (TDT) in PLINK 1.07 (Purcell et al., 2007) and by the parental-asymmetry tests (PAT, Weinberg, 1999). Finally, we explored the inclusion of a priori information on imprinted human genes using both data-base knowledge from http://www.geneimprint.com/ and information from Gregg et al. (2010a, b) to map imprinted mouse genes on homologous human genes.

Results: Even upon extending the marker density using 1000 genomes imputation, we observed no genome-wide significant imprinting signal at a level a of 5x10-8. Comparing the results of the stratified TDT and the PAT results were in some cases very different. Among the top 30 imprinting signals (according to PAT p-value) we observed no SNP in genomic regions (gene coding region ± 100kb) with previous evidence for imprinting.

Discussion: Our analyses demonstrate the 1000 genomes imputation had little effect compared to our previous findings. In contrast, the choice of the test statistic for addressing parent-of-origin effects in GWAS is important – at least for moderate sample sizes. We discuss our ongoing replication efforts.

A microdeletion on chromosome 1 of C57BL/6J mice results in a loss of function of Ifi202b which suppresses 11ß-hydroxysteroid dehydrogenase type 1 expression and development of obesity

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Complex diseases, such as obesity, are a result of the combination of genes and their interaction with the environment. The New Zealand Obese (NZO) mouse develops a polygenic disease pattern of obesity, insulin resistance, and dyslipoproteinemia closely resembling the human metabolic syndrome. In an outcross population of the NZO and the lean C57BL/6J (B6) mouse, we identified a major quantitative trait locus (QTL) for obesity (Nob3) on the distal mouse chromosome 1. Introgression of a 38 Mbp fragment (Nob3.38) - corresponding with the distal peak of the QTL - from NZO into the B6 background resulted in a markedly increased body weight, fat mass, lean mass, and a lower energy expenditure of NZO-allele carriers. For positional cloning of the responsible obesity gene, recombinant congenic lines (RCS) were generated and characterized, allowing defining a critical genomic interval comprising 43 genes. mRNA profiling and Western blotting indicated that Ifi202b, a member of the Ifi200 family of interferon inducible transcriptional modulators, was expressed in NZO-allele carriers but was undetectable in tissues of homozygous B6-allele carriers due to a microdeletion including the first exon and the 5'-flanking region of Ifi202b in B6. Transcriptome analysis of adipose tissue of RCS revealed a marked induction of 11ß-hydroxysteroid dehydrogenase type 1 (11ß-Hsd1) expression in mice expressing Ifi202b. Furthermore, siRNA-mediated Ifi202b suppression in 3T3-L1 adipocytes resulted in a significant inhibition of 11ß-Hsd1 expression, whereas an adenoviral-mediated overexpression of Ifi202b increased 11ß-Hsd1 mRNA levels. Expression of human IFI orthologues was significantly increased in visceral adipose tissue of obese subjects. We suggest that the disruption of Ifi202b in B6 is responsible for effects of the obesity QTL Nob3, and that Ifi202b modulates fat accumulation through expression of adipogenic genes such as 11ß-Hsd1.

Mutation screen in the GWAS derived obesity gene SH2B1 including functional analyses of detected variants

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Background: The SH2B1 gene (Src-homology 2B adaptor protein 1 gene) is a solid candidate gene for obesity. Large scale GWAS studies depicted markers in the vicinity of the gene; animal models suggest a potential relevance for human body weight regulation.
Methods: We performed a mutation screen in the SH2B1 coding sequence in 95 extremely obese children and adolescents. Detected variants were genotyped in independent childhood and adult study groups (up to 11,406 obese or overweight individuals and 4,568 controls). Functional implications on leptin mediated STAT3 signalling of the detected variants were analyzed in vitro.
Results: We identified two new rare mutations and five known SNPs (rs147094247, rs7498665, rs60604881, rs62037368 and rs62037369) in SH2B1. Mutation g.9483C/T leads to a non-synonymous, non-conservative exchange in the beta (ßThr656Ile) and gamma (γPro674Ser) splice variants of SH2B1. It was additionally detected in two of 11,206 (extremely) obese or overweight children, adolescents and adults, but not in 4,506 population-based normal-weight or lean controls. The non-coding mutation g.10182C/A at the 3' end of SH2B1 was only detected in three obese individuals. For the non-synonymous SNP rs7498665 (Thr484Ala) we observed nominal over-transmission of the previously described risk allele in 705 obesity trios (nominal p= 0.009, OR=1.23) and an increased frequency of the same allele in 359 cases compared to 429 controls (nominal p=0.042, OR=1.23). The obesity risk-alleles at Thr484Ala and ßThr656Ile/γPro674Ser had no effect on STAT3 mediated leptin receptor signalling in splice variants ß and γ.
Conclusion The rare coding mutation ßThr656Ile/γPro674Ser (g.9483C/T) in SH2B1 was exclusively detected in overweight or obese individuals. Functional analyzes did not reveal impairments in leptin signalling for the mutated SH2B1.

Genome-wide meta analysis across 270,000 individuals identifies seven sexually dimorphic variants associated with human anthropometric traits

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Gender differences in height, adiposity and fat distribution are well known. Previously, a sex-specific follow-up of a large scale meta-analysis of men and women combined revealed few sexually dimorphic genetic variants for human anthropometric traits.

To increase power to detect loci with sex-differences, we performed sex-specific genome-wide association meta-analyses of 46 studies (60,586 men, 73,137 women) and followed-up the results in 48 independent studies (62,395 men, 74,657 women) within the GIANT consortium.

Each study used an additive model to test up to 2.8M imputed SNPs for association with 9 phenotypes: height, weight, body mass index (BMI), waist and hip circumference (WC and HC), waist-hip ratio (WHR), the latter three with and without adjustment for BMI.

To maximize power, we pursued two parallel strategies to scan for sexually dimorphic variants of two classes: SNPs with marked association in only one sex (Single sex effect, SSE) and SNPs with association in both sexes but wit opposite effect direction (OED).

To detect SSE type of signals, we controlled sex-specific P-Values at 5 % false-discovery-rate (FDR) and as such selected 348 independent signals. A follow-up yielded 7 hits with significant (<5% FDR) sex-difference P-Values: (a) 6 women-specific loci for WHR adjusted for BMI (near GRB14/COBLL1, LYPLAL1, VEGFA, ADAMTS9, HSD17B4, PPARG) and one women-specific locus for WC adjusted for BMI (near MAP3K1).

Of particular interest is the PPARG region, which is a well-known target in type 2 diabetes treatments.

A second approach that was specifically tailored to detect OED type of SNPs did not yield any signals.

Our results highlight the importance of sex-stratified analyses and can help to better understand the genetics underpinning the sex-differences of human anthropometric traits.

Network-based SNP meta-analysis identifies joint and disjoint genetic features across common human diseases

Presenting Author: Hansjörg Baurecht

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Background: Genome-wide association studies (GWAS) have provided a large set of genetic loci influencing the risk for many common diseases. Association studies typically analyze one specific trait in single populations in an isolated fashion without taking into account the potential phenotypic and genetic correlation between traits. However, GWA data can be efficiently used to identify overlapping loci with analogous or contrasting effects on different diseases.

Results: Here, we describe a new approach to systematically prioritize and interpret available GWA data. We focus on the analysis of joint and disjoint genetic determinants across diseases. Using network analysis, we show that variant-based approaches are superior to locus-based analyses. In addition, we provide a prioritization of disease loci based on network properties and discuss the roles of hub loci across several diseases. We demonstrate that, in general, agonistic associations appear to reflect current disease classifications, and present the potential use of effect sizes in refining and revising these agonistic signals. We further identify potential branching points in disease etiologies based on antagonistic variants and describe plausible small-scale models of the underlying molecular switches.

Conclusions: The observation that a surprisingly high fraction (>15%) of the SNPs considered in our study are associated both agonistically and antagonistically with related as well as unrelated disorders indicates that the molecular mechanisms influencing causes and progress of human diseases are in part interrelated. Genetic overlaps between two diseases also suggest the importance of the affected entities in the specific pathogenic pathways and should be investigated further.

Meta-Analysis of Genome-wide Association Studies on Atopic Dermatitis Identifies Three Novel Risk Loci

Presenting Author: Hansjörg Baurecht

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Atopic dermatitis (AD) is a common chronic skin disease with high heritability and complex mode of inheritance. Apart from filaggrin (FLG), the genes influencing AD are largely unknown. We conducted a genome-wide association meta-analysis of 5,606 cases and 20,565 controls from 16 population-based cohorts and followed up the ten most strongly associated novel markers in a further 5,419 cases and 19,833 controls from 14 replication studies. Three SNPs among the ten were significant in the replication cohorts combined analysis, and met genome-wide significance in the discovery and replication cohorts combined. Two of these variants are located in genes which have been implicated in epidermal proliferation and differentiation, while one is located within the cytokine cluster on 5q31.1. Finemapping and conditional analyses indicated that there are two distinct signals at this locus, both of which were associated with transcript levels of IL13. We also replicated the FLG locus and two recently identified association signals at 11q13.5 and at 20q13.3. Our results underline the importance of both epidermal barrier function and immune dysregulation in AD pathogenesis.

Prioritization and Functional Characterization of Genes in Disease-Specific Networks

Presenting Author: Nadezhda T. Doncheva

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In recent years, many methods for candidate disease gene prioritization have been developed, some of which make use of functional annotations, exploit large-scale interaction data or integrate multiple data sources using statistical learning techniques. Since phenotypes can strongly differ in their genetic characteristics as well as in the amount of research dedicated to them, prioritization approaches should be tailored to specific phenotypes or groups of phenotypes. The available sources of biomedical knowledge can be used not only to prioritize candidate genes, but also to generate disease-specific networks that provide more insight into the functional characteristics of the underlying phenotypes.
In a case study on inflammatory bowel diseases, we generated networks of strong functional similarities between candidate genes based on their Gene Ontology annotations [2]. By analyzing and comparing the networks, we revealed the functional overlap of similar inflammatory phenotypes. Furthermore, we assessed the network connectivity of candidate genes using the Cytoscape plugin NetworkPrioritizer to select one disease-relevant gene per locus and build a disease-specific network. Finally, we combined the functional similarity networks with next-generation sequencing data to identify additional risk factors for inflammatory bowel diseases.

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Dense genotyping of known immune-mediated disease regions identifies nine new risk loci for primary sclerosing cholangitis

Presenting Author: Eva Ellinghaus

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Primary sclerosing cholangitis (PSC) is a severe liver disease of unknown etiology leading to fibrotic destruction of the bile ducts and ultimately the need for liver transplantation. Affected individuals are diagnosed at a median age of 30-40 years and suffer from an increased frequency of inflammatory bowel disease (60-80%) and autoimmune diseases (25%). Moreover, PSC patients are at increased risk of cancer of the bile ducts and colon. A 9-39-fold sibling relative risk indicates a strong genetic component in PSC risk. In addition to multiple strong associations within the HLA complex, recent association studies have identified genome-wide significant (P<5x10-8) loci at 1p36 (MMEL1/TNFRSF14), 2q13 (BCL2L11), 2q37 (GPR35), 3p21 (MST1), 10p15 (IL2RA) and 18q21 (TCF4). To identify additional PSC risk loci, we compared 3,789 European ancestry cases to 25,079 population controls genotyped using the Immunochip, assessing after quality control a total of 130,422 markers. The Immunochip is a genotyping array with densely spaced markers across 186 known disease loci from 12 immune-mediated diseases. In comparison to traditional genome-wide arrays, the greater marker density in known disease loci increases the ability to detect PSC associations within these key candidate genes. We identified 12 genome-wide significant associations outside the HLA complex, nine of which were novel (CD28, IL2/IL21, BACH2, SIK2, HDAC7, SH2B3, CD226, PKRD2 and 21q22.2). All associated variants are common (minor allele frequency > 0.17) and of moderate effect sizes (odds ratios between 1.15 and 1.33). By incorporating pleiotropy with seven diseases clinically co-occurring with PSC in the analysis, we found suggestive evidence (FDR<0.001 and P<5x10-5) for 33 additional PSC risk loci. This study further completes the genetic risk map of PSC and yields additional insights into the relationship between PSC and other immune-mediated diseases.

Towards blood-born microRNA biomarkers for inflammatory bowel disease

Presenting Author: Georg Hemmrich-Stanisak

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Invasive methods, such as endoscopy or colonoscopy are still the gold standard for diagnosis and evaluation of Crohn's disease (CD) and ulcerative colitis (UC). Due to their stability in tissues and body fluids, such as peripheral blood, differential signatures of microRNAs (miRNAs) could serve as new noninvasive biomarkers to predict and discriminate these two major subphenotypes of chronic inflammatory bowel disease (IBD). Furthermore, identification and subsequent in-depth study of selected IBD-linked miRNAs may add significant knowledge to the overall understanding of this complex disease and enable for new treatment options. In this study, we determined the expression profiles of 863 miRNAs by array analysis of 114 blood samples (40 CD, 36 UC, 38 healthy controls) to generate phenotype-specific miRNA signatures. Implementing a series of SVM-based machine-learning algorithms for classification we could identify phenotype-specific miRNA-signatures using a randomly split sample-set for feature selection, parameter training, model evaluation and replication at a ratio of 2:3:3:2. Furthermore we tested whether filtering according to deregulated miRNA-expression could reduce the number of necessary calculation steps. Applying a threshold of maximum 100 features per signature resulted in miRNA-signatures of 24 (CD) and 3 (UC) deregulated miRNAs that separated cases from controls with a balanced accuracy of 100% and 82%, respectively. To discriminate both subphenotypes a signature of only 2 differentially expressed miRNAs is required (balanced accuracy 81%). For validation of our results we plan to replicate the approach using an independent IBD-cohort. In addition an observer-blinded study will prove the predictive potential of the resulting phenotype-specific miRNA signatures. To gain further insights into possible biological functions of phenotype-specific miRNAs we currently try to narrow down the manifold possible miRNA target genes to disease-associated cellular pathways.

RNAi screening identifies FRMPD2: a scaffolding protein involved in basolateral NOD2 signaling

Presenting Author: Simone Lipinski

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The cytosolic pattern recognition receptor NOD2 is the prototypical member of the NOD-like receptor (NLR) family and a key player in host defense. NOD2 detects bacteria-derived muramyl dipeptide (MDP) and activates pro-inflammatory signaling cascades like the NF-kappaB pathway. In the present study, we used a systematic siRNA screen comprising 7784 genes (druggable genome), to uncover relevant modulators of NOD2-dependent NF-kappaB signaling. Using a NF-kappaB-dependent luciferase reporter assay as primary readout we identified a set of 20 positive NF-kappaB regulators including the known pathway members RIPK2, RELA and BIRC4 (XIAP) and the hitherto unknown activator FRMPD2 (FERM and PDZ domain containing 2). We found that FRMPD2 directs NOD2-mediated MDP recognition to the basolateral membrane of polarized intestinal epithelial cells (IEC) by physically interacting with leucine-rich repeats (LRR) of NOD2. In addition, FRMPD2 facilitates membrane recruitment of RIPK2, the adaptor kinase of NOD2, resulting in the formation of a functional multiprotein NOD2 signalosome. We show that genetic truncation of the NOD2 LRR domain (L1007insC), which is associated with Crohn's disease, impairs the interaction with FRMPD2, and that intestinal inflammation leads to down-regulation of FRMPD2. These results for the first time suggest a structural mechanism where FRMPD2 acts as a membrane scaffolding complex providing a spatial control mechanism for NOD2-mediated immune responses.

Polymorphisms in SLC1A2 are associated with essential tremor.

Presenting Author: Kuhlenbäumer G

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Objective: Sporadic, genetically complex essential tremor (ET) is one of the most common movement disorders and may lead to severe impairment of the quality of life. Despite a high heritability the genetic determinants of ET are largely unknown. We performed the second genome wide association study (GWAS) for ET to elucidate genetic risk factors of ET.
Methods: Using the Affymetrix® Genome-Wide SNP Array 6.0 (1000K) we conducted a two-stage GWAS in a total of 990 cases and 1537 controls from Europe to identify genetic variants associated with ET.
Results: We discovered association of an intronic variant of the main glial glutamate transporter (SLC1A2) gene with ET in the first stage sample (rs3794087, P = 6.95 × 10(-5), odds ratio (OR) = 1.46). We verified the association of rs3794087 with ET in a second stage sample (P = 1.25 × 10(-3), odds ratio (OR) = 1.38). In the subgroup analysis of patients classified as definite ET, rs3794087 obtained genome-wide significance (P = 3.44 × 10(-10), odds ratio (OR) = 1.59) in the combined first and second stage sample. Genetic fine mapping using non-synonymous SNPs and SNPs in high linkage disequilibrium with rs3794087 did not reveal any SNP with a stronger association with ET than rs3794087.
Conclusions: We identified SLC1A2 encoding the major glial high affinity glutamate reuptake transporter in the brain as a potential ET susceptibility gene. Acute and chronic glutamatergic overexcitation is implied in the pathogenesis of ET. SLC1A2 is therefore a good functional candidate gene for ET.

Dense genotyping of six atopic dermatitis and 180 autoimmune risk loci in 2,425 atopic dermatitis patients

Presenting Author: Elke Rodríguez (1)

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Atopic dermatitis (AD), or eczema, is one of the most common chronic inflammatory skin diseases, with a polygenic, multifactorial nature. To date, genome-wide association studies have established six susceptibility loci with genome-wide significance ($P<5\times10^{-8}$). However, the causal variation at these loci remains unknown. To better define risk variants and identify additional susceptibility loci previously implicated in other autoimmune (AI) diseases, we performed a fine-mapping and association study using 2,425 German AD cases and 5,449 German population controls. All samples were genotyped on the ImmunoChip, a custom Illumina Infinium High-Density array containing 196,524 polymorphisms (195,806 SNPs and 718 small insertions/deletions) across 186 distinct AI risk loci, including the six known AD susceptibility loci and the HLA region. At these loci, the array contains all known SNPs in the dbSNP database, from the 1000 Genomes Project (release of February 2012), and from other AI disease resequencing efforts, therefore providing a powerful means of fine-mapping known AI loci.

Following quality control, 128,830 polymorphic markers (SNPs with minor allele frequency > 1%) were available for association analysis, with an individual call rate > 99.9%. Genotypes were called using Illumina's GenomeStudio GenTrain 2.0 algorithm. Principal component analysis revealed no marked differences in ancestry between cases and controls. We observed that 132 SNPs within non-HLA risk loci reached genome-wide significance ($P<5\times10^{-8}$) in the screening phase. For each locus, the possibility of the existence of multiple independent association signals will be investigated. In addition, we selected the most strongly associated SNPs (n=39) with $P<10^{-4}$ from each associated locus for replication analysis in independent case-control collections. The final results will be presented at the conference.

Extracellular Cathepsin K is protective against chronic intestinal inflammation in mice and involved in host-microbe homeostasis

Presenting Author: Konrad Aden, Simone Lipinski

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BACKGROUND & AIMS: Cathepsin K is a lysosomal cysteine protease known for its importance in bone resorption. Recently, a role for cathepsin K in chronic inflammatory responses became evident upon the demonstration that cathepsin K-deficient (Ctsk-/-) mice are highly resistant to experimental autoimmune arthritis and encephalomyelitis. Here, we address the relevance of cathepsin K in the intestinal immune response during chronic intestinal inflammation.

METHODS: Chronic colitis was induced by three cycles of 2% DSS (dextran sodium sulfate) treatment in Ctsk-/- and WT mice. Mice were assessed for disease severity, histopathology and endoscopic appearance. Furthermore, DSS-exposed Ctsk-/- mice were subjected to rectal administration of recombinant cathepsin K. Changes in intestinal microbial signatures were assessed by using real-time quantification of fecal and colonic biopsy samples and 16S rRNA microbial fingerprinting by 454 sequencing.

RESULTS: Here, we demonstrate a protective role of cathepsin K using a Ctsk-/- mouse colitis model. Dissecting the underlying mechanisms we found that cathepsin K is expressed by intestinal goblet cells and present in the mucin layer. Also, intestinal microbiota differed significantly between Ctsk-/- and WT mice. We found that cathepsin K exerts a direct anti-microbial activity, which potentially explains the altered intestinal microbiota observed in Ctsk-/- mice. Notably, rectal administration of recombinant cathepsin K in DSS-treated Ctsk-/- mice ameliorates the severity of intestinal inflammation.

CONCLUSIONS: Our data assign a key role to extracellular cathepsin K for maintenance of microbial colonic communities. We suggest that topical administration of cathepsin K could lead to new avenues for the development of therapies restoring intestinal homeostasis in IBD.

Whole Exome Sequencing of 42 Crohn's Disease Patients and Identification of Rare Variants associated with Crohn's Disease

Presenting Author: David Ellinghaus

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Crohn's disease (CD) is one of the most important subphenotypes of common idiopathic inflammatory bowel disease (IBD) with high relative risk of siblings to affected individuals (λs ~ 25-42). A recent meta-analysis of six distinct genome-wide association studies (GWAS) established 71 CD susceptibility loci as genome-wide significant. However, the 71 loci described so far account for only a limited amount (~ 23.2%) of CD heritability. This suggests that the more common (>5% allele frequency) GWAS tag SNPs are accompanied by many large effect low-frequency variants. High-throughput sequence capture methods coupled with next generation sequencing (NGS) technologies offer the opportunity to extract almost the complete variation in regions of interest which may help to elucidate the missing genetic contribution to disease susceptibility, in our case CD.

We captured the exomes of 42 german CD patients, one HapMap trio and 3 german unrelated healthy control individuals by means of the NimbleGen 2.1M Human Exome Array, and subsequently sequenced on the Illumina Genome Analyzer (GA) platform. In total, 39,370 megabases (Mb) of mappable sequence was generated and was mapped to target region for our 48 individuals, with an average read depth on target of 24.77 per individual exome. On average, approximately 96.92% of targeted bases were successfully covered. We identified 117,957 SNPs in our 48 individuals, including 59,076 coding and splice site SNPs (cSNPs + ssSNPs). On the basis of existing filtering strategies that had been demonstrated to work well for monogenic disorders, we developed a strategy approach that incorporates association results from the recent CD meta-analysis in combination with previously developed in silico mutation effect prediction algorithms. Selected rare variants were then subjected to a replication phase using large and independent case-control collections comprising 9,348 CD cases, 2,868 ulcerative colitis cases, and 14,567 healthy control individuals.

Exome sequencing for early-onset Crohn's disease

Presenting Author: Britt-Sabina Petersen

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Crohn's disease (CD) is a complex chronic inflammatory bowel disease (IBD). A variety of genetic and environmental factors likely play a role in causing CD. Genome-wide association studies (GWAS) and meta-analyses have so far identified 71 susceptibility loci for CD. These, however, explain only 23% of the heritability so far. To identify further sources of heritability, other approaches are necessary, for example the identification of rare susceptibility variants with presumably higher penetrance by systematic resequencing studies. We have carried out exome sequencing for several pedigrees with Crohn's disease for finding novel candidate genes involved in disease etiology.
Amongst others we sequenced the exomes of a 3-year-old boy who presented with early-onset IBD during his first year and his healthy parents and identified a single de novo mutation as the cause: a novel hemizygous nonsense SNP in the X-linked inhibitor of apoptosis gene (XIAP). Mutations in XIAP are known to cause X-linked lymphoproliferative disease type 2 (XLP2) and have once previously been reported to cause early-onset IBD in a male patient. Our results indicate that mutations in XIAP are not only associated with XLP2 but also may play an important role in early-onset IBD cases.

The Genes in Irritable Bowel Syndrome Research Network Europe (GENIEUR)

Presenting Author: Beate Niesler

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Background: GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe) is a pan-European interdisciplinary network aiming to identify genetic factors contributing to Irritable bowel syndrome (IBS). More than 15 % of the population worldwide suffer IBS. IBS is a functional gastrointestinal (GI) disorder is characterized by a high comorbidity with psychiatric conditions such as anxiety and depression as well as with pain syndromes.
Methods: GENIEUR is focusing on the following objectives: Standardization and harmonization of criteria for case control definition and patient recruitment and characterization, establishing a phenotyping tool, creation of a database, collecting data such as exposure to environmental factors (germs, infection, nutrition), establishment of a biobank (blood, tissue, stool, urine) not only for genetic studies but also for analyses of functional / phenotypic consequences of coined genetic factors.
Results: The following working groups (WGs) have been established: WG 1) Establishment of a gold standard for patient recruitment and characterization, WG 2) Definition of quantitative traits as intermediate phenotypes, WG 3) Genetics: Molecular genetics and epigenetics and WG 4) Microbiomics. Experts working from complementing disciplines such as gastroenterology, psychiatry, physiology, pathology, immunology, nutrition as well as genetics, microbiology, bioinformatics and molecular biology join forces in order to address the major objectives.
Discussion: GENIEUR represents an excellent platform for unifying and harmonizing research strategies, which is a prerequisite to nail down crucial factors involved in IBS. With this Action, Europe-wide collaboration will be facilitated with a significant impact on IBS research all over Europe by networking of renowned scientific experts from different disciplines from different countries.

National Genome Research Network

Poster Presentation Abstracts

Symposium IV

Genomics of Common Disease II

MTSS1 inactivation is associated with increased migration and invasion of glioma cells

Presenting Author: Andreas Waha

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In a genome-wide methylation analysis, the metastasis suppressor-1 (MTSS1) gene was identified as a novel gene hypermethylated in gliomas. MTSS1 has been associated with cross linking and membrane attachment of actin filaments. Here we investigate MTSS1 methylation, allelic loss and expression in 63 diffuse high grade gliomas and 6 glioma cell lines by pyrosequencing, realtime RT-PCR and IHC. In addition we transfected glioma cells with MTSS1-EGFP to study the localization as well as the functional impact of MTSS1 protein in vitro.

MTSS1 hypermethylation was frequently found in anaplastic astrocytomas (AAIII) and secondary glioblastomas (sGBM-IV) where it correlated with IDH1 mutation status (P=0.0001). Epigenetic silencing of MTSS1 by DNA methylation was confirmed by treatment of glioma cell lines with the demethylating agent 5-aza 2' deoxycytidine. Although 30% of investigated glioblastoma tissues showed reduced MTSS1 mRNA, no significant correlation between MTSS1 methylation and transcript levels was found. Allelic loss was identified in 2/9 and 2/6 of informative AAIII and sGBM-IV respectively, whereas only 2/22 investigated primary glioblastomas showed loss of MTSS1 alleles.

MTSS1-EGFP transfected glioma cells LN229 and U87MG showed staining of focal contact structures suggesting a role of MTSS1 in cellular adhesion. In line with this assumption, a significant negative impact of MTSS1 protein expression on the migration and invasion of glioma cells was confirmed in scratch assays and trans-well chamber analyses. Kaplan Meier analyses of REMBRANDT glioma data sets show a significant correlation of MTSS1 expression with patient survival (P=0.00017).

We conclude that inactivation of MTSS1 may contribute to the molecular pathology of diffuse high grade gliomas by reducing attachment and enhancing migration/invasion of glioma cells which may contribute to a shorter survival of glioma patients.

Association of TET1 nuclear exclusion with loss of 5-hydroxymethylcytosine in IDH1 wild-type gliomas

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The identification of isocitrate dehydrogenase 1(IDH1) gene mutations in gliomas inspired various studies exploring the molecular consequences and the clinical implications of such alterations. The Cancer Genome Atlas Research Network uncovered a CpG island methylator phenotype (G-CIMP) in glioblastomas that was associated with IDH1- mutations. Mutant IDH1 protein produces the onco-metabolite 2-hydroxyglutarate that inhibits a-ketoglutarate dependent oxygenases, e.g. TET enzymes involved in the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine. We investigated 60 gliomas for the presence of 5-hydroxymethylcytosine, 5-methylcytosine content, TET1 expression and IDH1 mutation to gain insight into their relationship on the histological level. 61% of gliomas revealed no immunoreactivity for 5-hydroxymethylcytosine and no correlation was observed between IDH1 mutations and loss of 5-hydroxymethylcytosine. Interestingly, expression of TET1 showed remarkable differences with regard to overall protein levels as well as subcellular localization. We found a highly significant (P= 0.0007) correlation between IDH1 mutations and nuclear accumulation of TET1 but not with loss of 5hmC. 70% of 5-hydroxymethylcytosine negative gliomas showed either exclusive or dominant cytoplamic expression or no detectable TET1 protein (P= 0.0122). Methylation of MGMT and DUSP4/MKP2 was more abundant in gliomas showing nuclear expression of TET1 and harboring mutant IDH1 alleles, but no correlation between global 5-hmC levels and methylation of these genes was observed.

A common variant of melanoma inhibitory activity 2 modifies chemoresponsiveness by interfering with the internal endoplasmic reticulum stress response

Presenting Author: Bo Kong

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Pancreatic ductal adenocarcinoma (PDAC) is an extremely aggressive and chemotherapy-resistant cancer. The HNF1 homeobox A (HNF1A) region has been identified as a novel PDAC susceptibility locus. One target molecule of HNF1A is melanoma inhibitory activity 2 (MIA2) - a secreted protein which locates at the endoplasmic reticulum (ER) exit site, which interacts with coat protein complex-II (COPII), and thus facilitates protein secretion. Here, we identified a common germline variant of MIA2-I141M associated with a secretory defect in PDAC cells. Compellingly, PDAC patients carrying the I141M variant survived longer after tumor resection than those carrying the wild-type (median survival: 28 vs. 18 months, p=0.02). However, the survival benefit of I141M was only restricted to those patients who received adjuvant chemotherapy, indicating that the I141M variant affected chemoresponse. Consistently, PDAC cell lines expressing the I141M variant were highly sensitive to gemcitabine in vitro. Gene expression profiling revealed that the I141M variant was associated with an increased expression of genes involved in the unfolded protein response (UPR; especially the ERN1/XBP1 arm) in tissues and cell lines. In particular, increased ERN1 expression was responsible for increased sensitivity to gemcitabine caused by the MIA2-I141M variant. These findings are of clinical significance because they hold the promise to (sub-)classify PDAC patients into groups that are more likely to benefit from (adjuvant) chemotherapy.

Multi-objective parameter selection for classifiers

Presenting Author: Christoph Müssel, Ludwig Lausser

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Choosing the proper values for parameters is an essential step in classifier training. Often, such parameters are set according to rules of thumb. Parameter tuning is an automated way of adapting parameters. In many cases, different concurrent criteria can be employed to rate the quality of a model (e.g. sensitivity and specificity). In this scenario, different trade-offs of the criteria may exist.
The ultimate decision which trade-off is suited best may be made by a human expert depending on additional external knowledge (e.g. high sensitivity for screening procedures, high specificity for confirmatory tests).
We establish a framework for multi-objective classifier parameter selection on the basis of Pareto dominance. The methodology covers sampling strategies for candidate parameter configurations and also includes evolutionary optimization.
A generic implementation of the approach is freely available in the TunePareto R package and is capable of tuning arbitrary classifiers.

References

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On the Utility of Partially Labeled Data for Classification of Microarray Data

Presenting Author: Ludwig Lausser

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Microarrays are standard tools for measuring thousands of gene expression levels simultaneously. They are frequently used in the classification process of tumor tissues. In this setting a collected set of samples often consists only of a few dozen data points. Common approaches for classifying such data are supervised. They exclusively use categorized data for training a classification model. Restricted to a small number of samples, these algorithms are affected by overfitting and often lack a good generalization performance. An implicit assumption of supervised methods is that only labeled training samples exist. This assumption does not always hold. In medical studies often additional unlabeled samples are available that can not be categorized for some time (i.e., ”early relapse” vs. ”late relapse”). Alternative classification approaches, such as semi-supervised or transductive algorithms, are able to utilize this partially labeled data. Here, we empirically investigate five semi-supervised and transductive algorithms as ”early prediction tools” for incompletely labeled datasets of high dimensionality and low cardinality. Our experimental setup consists of cross-validation experiments under varying ratios of labeled to unlabeled examples. Most interestingly, the best cross-validation performance is not always achieved for completely labeled data, but rather for partially labeled datasets indicating the strong influence of label information on the classification process, even in the linearly separable case.

Knockdown of kinesin motor protein Kif20a leads to growth inhibition in pancreatic ductal- and neuroendocrine-cancer cells.

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Aim: To characterize potential molecular drug targets in pancreatic cancer, we investigated Kif20a, which belongs to the kinesin superfamily involved in trafficking of molecules and organelles.
Methods: Detection of Kif20a as a druggable candidate was made by: combined evaluation of various high-throughput gene analysis panels. In vitro analysis were made in pancreatic ductal adenocarcinoma (PDAC) and neuroendocrine cancer (NEC) cell lines using quantitative realtime-PCR, immunohistochemisty, immumofluorescence, immunoblot methods, MTT- and invasion assay.
Results: Immunohistochemical analysis of paraffin embedded pancreatic tumor samples showed a stronger staining in cancer than in healthy pancreatic tissues. Stronger immunostaining was also observed in several altered acinar cell cluster in chronic pancreatitis.
Immunofluorescence analysis of pancreatic ductal- and neuroendocrine cancer cells lines showed nuclear and cytoplasmic localization. mRNA and protein expression of Kif20a was comparable in three PDAC and three NEC cell lines. Knockdown of Kif20a with small interfering RNA molecules leads to 35-40% and 15-30% reduction of proliferation in PDAC and NEC cell lines, respectively. Invasion capacity of PDAC cells was suppressed by 40-50% after Kif20a knock down.
Conclusion: With an upregulation of more than 10-fold in pancreatic cancer cells, Kif20a appears as a likely candidate for development of drugs to treat pancreatic ductal adenocarcinoma at the molecular level.

Molecular markers in endobronchial epithelial lining fluid: Early detection of lung cancer and other severe lung diseases

Presenting Author: Ruprecht Kuner

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Bronchoscopic microsampling (BMS) and the identification of disease biomarker in endobronchial epithelial lining fluid (ELF) represents a less-invasive diagnostic approach for the early detection of severe lung diseases. For example, early detection of lung malignancies could enable a timely intervention and subsequently a reduction of the high mortality rate of this disease. We investigated whether transcript analysis in ELF may be useful for a definitive preoperative diagnosis.

ELF was collected from subsegmental bronchi close to the indeterminate pulmonary nodule detected by computer tomography, and from the contralateral lung. Diagnosis was confirmed by transbronchial biopsy or surgery. In a proof-of-principle study, we checked the feasibility of the BMS/ ELF approach, complication rate, and the ability to quantify gene transcripts from a minimal number of cells. So far, 178 ELF samples were collected from lung cancer patients and control individuals with benign lung diseases. All patients underwent BMS without complications. A microarray-based screening study narrowed down gene transcripts associated with a malignant phenotype. Several potential biomarkers were validated by qRT-PCR in an independent patient cohort. Of note, gene expression analysis could be reliably applied to all ELF samples. Tenascin-C (TNC), involved in lung tumorigenesis and lung metastasis, was found as a promising transcript biomarker whose expression was increased in ELF samples close to pulmonary nodules with malignant diagnosis. Combined analysis of TNC expression and the nodule size improved the prediction of malignancy in this patient cohort. Further ELF samples are currently analyzed to check for TNC splice variant expression, which might improve the specificity of the diagnosis. Furthermore, in the German Center of Lung Research, we aim to expand the BMS/ ELF method for other severe lung diseases like COPD and fibrosis.

The role of a novel microRNA cluster, C19MC, in breast cancer

Presenting Author: Aoife Ward

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microRNAs (miRNAs) are a novel class of small non-coding RNAs which have the ability to post-transcriptionally regulate proteins. Recently, miRNAs have been shown to be involved in the acquired resistance to a number of cancer therapies. We developed in vitro models of acquired resistance to tamoxifen and trastuzumab and employed genome wide arrays to analyze changes in miRNA and gene expression.
Interestingly, the miRNA cluster C19MC, which harbours around 50 mature miRNAs, was upregulated in resistant cells. Studies have suggested that this cluster is epigenetically regulated. Indeed, upon treatment with a demethylating agent, we observed that members of C19MC were re-expressed in breast cancer cells. We are currently investigating whether this cluster is regulated by DNA methylation.
We chose one of the top upregulated miRNAs in the cluster and found that it was involved in resistance to both tamoxifen and trastuzumab. By combining gene expression data and prediction algorithms, we identified important tumor suppressor genes as potential target genes of the miRNA.
In addition, the miRNA had an oncogenic function in breast cancer as it induced cell cycle progression as well as resistance to apoptosis. Finally, using publically available data, we could show that patients with a high expression of this miRNA had a poorer survival.
In summary, we have identified a miRNA which serves as an oncomir and is capable of co-regulating many processes in breast cancer. Using inhibitors of may serve as a novel therapeutic approach to combat resistance to therapy as well as proliferation and evasion of apoptosis in breast cancer.

Murine cytomegalovirus infection of cultured mouse cells induces expression of miR-7a

Presenting Author: Alexandra Dittmer

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Mouse Cytomegalovirus has been described to generate large numbers of viral miRNAs during lytic infection and was therefore used to analyze the impact of viral miRNAs on the host cell small RNA system as well as to check for sorting of viral small RNAs into specific Ago-proteins. Deep sequencing analysis of MCMV infected cells revealed that viral miRNAs represent only app. 13% of all detected miRNAs. All previously described MCMV miRNAs with the exception of miR-m88-1* were confirmed and for the MCMV miR-m01-1 hairpin an additional miRNA, designated miR-m01-1-3p, was found. Its presence was confirmed by qPCR and Northern Blot. Deep sequencing after RISC IP with antibodies specific for either Ago1 or Ago2 showed that all MCMV miRNAs are loaded into both RISC complexes. The ratio of MCMV to mouse miRNAs was not increased after immunoprecipitation of Ago-proteins. Viral miRNAs therefore do not overwhelm the host miRNA processing system nor are they preferentially incorporated into RISC.
We found that 3 mouse miRNAs showed an altered expression due to MCMV infection. Downregulation of miR-27a, as previously described, could be confirmed. In addition, miR-26a was downregulated and an upregulation of miR-7a dependent on viral protein expression and DNA replication could be observed. Transfection of cells with miR-7 mimics or inhibitors had no effect on viral growth, indicating that miR-7 induction has no effect on viral replication in cell culture.

Kaposi's sarcoma herpesvirus (KSHV) microRNAs regulate apoptosis by targeting caspase 3

Presenting Author: Felicia Wagner

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic gamma-herpesvirus which causes Kaposi's sarcoma and atypical lymphomas. It encodes twelve micro (mi)RNAs, which are expressed from a latent transcript that is upregulated during the lytic stage. Since previous studies reported that KSHV is able to inhibit apoptosis during latent infection, the involvement of viral miRNAs in this process has been tested. In fact, both HEK293 epithelial cells and DG75 B-cells stably expressing KSHV miRNAs were found to be protected from apoptosis. Potential cellular targets that were significantly down-regulated upon KSHV miRNAs expression were identified by microarray profiling, and caspase 3 (Casp3), a critical factor for the control of apoptosis, was one of the genes that was found to be regulated. Casp3 could be validated as miRNA target by luciferase reporter assays, quantitative PCR and western blotting. Using site-directed mutagenesis, three KSHV miRNAs, miR-K12-1, 3 and 4-3p, were identified to be responsible for the targeting of Casp3. Specific inhibition of these miRNAs in KSHV-infected cells resulted in increased expression levels of endogenous Casp3 and enhanced apoptosis. In summary, these results suggest that KSHV miRNAs directly participate in the inhibition of apoptosis by the virus, and are thus likely to play a role in KSHV-induced oncogenesis.

Identification of targets of murine gammaherpesvirus 68 (MHV-68) encoded miRNAs by combining bioinformatics and various experimental approaches

Presenting Author: Martin Strehle

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The murine gammaherpesvirus 68 (MHV-68) serves as a small animal model for infections with the human gammaherpesviruses Kaposi's sarcoma-associated herpesviruses (KSHV) and Epstein-Barr virus (EBV) which are associated with a variety of tumors. Both KSHV and EBV encode miRNAs which are proposed to play an important role in pathogenesis. As in vivo studies with KSHV and EBV are limited to clinical investigation of the infection, MHV-68 might represent a useful model to study in vivo functions of gammaherpesvirus miRNAs both during the lytic and latent phase of infection. We first aimed to identify targets of the MHV-68 miRNAs. For this purpose, we used several approaches: i) immunoprecipitation of RISC-complexes followed by microarray (RIP-Chip) or nanostring (RIP-nanostring) analysis of the RISC-bound miRNA targets; ii) comparison of global gene expression profiles of cells infected with wildtype or mutant MHV-68 lacking miRNAs, and iii) bioinformatical prediction of miRNA targets using „Targetscan" (www.targetscan.org). Each approach resulted in a list of candidate targets. Merging the three lists resulted in 14 most relevant targets. The putative target sequences of selected candidate genes were cloned into luciferase reporter plasmids and tested for their biological relevance by luciferase assays. To improve the confidence of the targets identified, we additionally plan to apply methods like Argonaute HITS-CLIP (High-Throughput Sequencing of RNAs from in vivo Cross-Linking and Immuno-Precipitation). Our data will aid to better understand the roles of virally encoded miRNAs during gammaherpesvirus infection.

Genome-wide identification of DNA methylation changes upon Helicobacter pylori infection

Presenting Author: Sabrina Janßen

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Aberrant DNA methylation patterns are a hallmark of cancer. DNA methylation can silence the expression of genes involved in fundamental pathways that ultimately lead to cancer. The most important factor in gastric carcinogenesis is H. pylori infection. In the last years, several publications point to a connection between H. pylori infection and the development of gastric cancer via epigenetic modifications on a gene and chromatin level. However, a genome wide overview of infection-induced modifications is still missing.
Methyl-DNA immunoprecipitation (MeDIP) assays, in combination with microarray hybridization techniques, are excellent tools for identifying methylated CpG-rich sequences. We captured methylated DNA using a methyl-CpG binding domain-based (MBD) protein and hybridized the resulting DNA proportion on a Nimblegen CpG Island-Plus-Promoter Array. We found that H. pylori alters the DNA methylation patterns in gastric epithelial cells. With our approach we were able to identify differentially methylated regions (DMRs). We further performed empirical analyses and found methylation differences for promoter and CpG rich regions.
These results represent the first genome wide methylation analysis of H. pylori infected cells. They will help to understand the strategies of pathogens in modifying DNA methylation patterns and also to identify targets for therapeutic treatment and diagnostic biomarkers, particularly in relation to cancer.

Screening and validation for transcription factors influencing EGF-induced migration

Presenting Author: Kirti Sharma

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Migration and invasion belong to the hallmarks of cancer leading to metastasis and dissemination of cancer. This is the major cause of high mortality rate associated with cancer in general and breast cancer in specific. MCF-10A cells are non-tumorigenic breast epithelial cells. They are known to undergo a change from resting to migratory phenotype in presence of Epidermal Growth Factor (EGF). In our current study, we aim to identify transcription factors (TFs) responsible for this complex phenotypic switch. To address this question, we performed a focused RNAi screen with 183 TFs based on microarray expression data and relevance in breast cancer. Effects of candidate transcription factor knockdown on EGF-induced migration of MCF10A cells were analyzed applying an automated wound healing assay. The screen has identified 1 accelerator and 2 inhibitors of cell migration which could be validated using independent assays. Our goal now is to unravel their mechanistic mode of action to be able to better explain the phenomenon of migration in context of EGFR signaling pathway.

Protein-protein interactions in human pluripotent stem cell-derived neural stem cells and their neuronal progeny

Presenting Author: Jonas Doerr

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Protein interaction studies represent a powerful tool to study cell signaling cascades, cell-cell interactions as well as principles of signal transduction mechanisms. Up to today, the majority of human interactome studies were conducted based on overexpression paradigms in tumor cell lines. Commonly encountered problems in this context are unspecific interactions due to supra-physiological protein expression levels, the use of transformed cells and a non-tissue specific proteome. Here we used pluripotent stem cell-derived neural stem cells (lt-NES®; Koch et al., 2009) as a somatic stem cell population, which exhibits extensive self-renewal, clonogenicity and stable neurogenesis. The introduction of GFP-tagged proteins via bacterial artificial chromosomes (BAC; Poser et al., 2008) permitted the derivation of large numbers of polyclonal cell populations (pools) with faithful protein expression in more than 90% of the cells, average integration rates of one locus per genome as well as correct size and compartmentalization of the tagged. Using protein-protein interaction studies with a label-free, quantitative affinity purification–mass spectrometry approach (Hubner et al., 2010) we identified several known complexes as well as novel interaction candidates of these proteins. The system is sufficiently sensitive to report changes in protein expression levels and compartmentalization during lt-NES® cell differentiation and after exposure to extrinsic factors such as inhibitors of proliferation and modulators of cell signaling. Furthermore, we were able to transfer this technique to iPS cell-derived lt-NES® cells. Our data suggest that protein tagging in PSC-derived lt-NES® cells and their neuronal progeny represents an efficient approach for studying protein-protein interactions in human neural cells both in normal and neurodegenerative contexts.

German Mouse Clinic 2 – Behaviour and Neurogenesis

Presenting Author: Lillian Garrett

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As part of the genome-environment interaction platform, German Mouse Clinic 2, we look at the role played by neurogenesis in behaviour and how this relationship is altered in disease. Adult neurogenesis occurs in two brain regions: the subventricular zone along the walls of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus. The precise functional role of these newly generated cells is not known. Employing a combination of environmental (exercise, psychological stress) and genetic (doublecortin (DCX)-Cre mutant mice) manipulations, we are looking at the fundamental role played by neurogenesis in both emotion-related and cognitive behaviours in mice. Using voluntary wheel running as a means to increase the rate of neurogenesis, we have examined the correlation between exercise-increased neurogenesis and anxiety-like behaviour in tests such as the open field and the light/dark box. We have also performed an in-depth behavioural phenotyping (from anxiety- and depression-related behaviour to cognition) of the tamoxifen-inducible doublecortin-Cre mutant mice, where neurogenesis is decreased through targeting the doublecortin protein. Implementing further genetic models, we are examining the connection between altered levels of neurogenesis and behaviour in disease. Subsequent to a comprehensive behavioural phenotyping, we are correlating the level of neurogenesis to the behavioural profile of these mice. The aim of these investigations is to determine whether the altered level of neurogenesis underlies the observed behavioural changes.

Fgf9 Signaling Regulates Expression of Crystallin Genes and Lim2 during Lens Fiber Differentiation

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Aims: Fibroblast growth factor (Fgf) signaling plays a crucial role in many developmental processes. Among the Fgf pathway ligands, Fgf9 participates in maturation of various organs and tissues including the eye. We recently established the novel Fgf9Y162C mutant mouse line Aca12 in a dominant N-ethyl-N-nitrosourea (ENU) screen for eye-size abnormalities. Aca12 was originally identified because of its significantly reduced lens thickness that goes along with age-related cataract formation. In this study, we investigated putative consequences of Fgf9Y162C on cell proliferation and differentiation during lens development.
Methods: Relative gene expression in ocular tissues of E12.5 mouse embryos was determined by Real-time PCR. Lens size and transparency of four-month-old mice was analysed by optical low coherence interferometry and Scheimpflug imaging, respectively.
Results: Our gene expression studies showed that the transcription factors Pax6, Six3, and Mab21/1 of the lens cell proliferation pathway are regularly expressed in ocular tissues of homozygous Aca12 embryos. Concerning lens fiber differentiation, we found a regulation of Sox2 (reduced to 50%) and Prox1 (increased to 318%) that resulted in a down regulation of the Sox2/Prox1 target genes CrygD (crystallin gamma D; reduced to 64%) and Lim2 (lens intrinsic membrane protein 2; reduced to 76%).
We further investigated lens size and density of adult Aca12/Cryba2S47P and Aca12/Lim2C51R compound mutant mice. Mean lens thickness was 6.6-22.1% decreased compared to the situation in Aca12, Cryba2S47P, and Lim2C51R single mutants. Moreover, we found an increase of mean lens density between 12.6% and 39.8%. These data further established an interaction between Fgf9 signaling and expression of Cryba2 and Lim2 in the developing lens.
Conclusions: Taken together, the results of our study demonstrate that Fgf9 signaling plays a role in regulating components of the lens fiber differentiation pathway.

New test assays for metabolic phenotyping of mouse mutants in the German Mouse Clinic

Presenting Author: Jan Rozman

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The Energy Metabolism Screen of the German Mouse Clinic (GMC) focuses on gene functions in energy balance. Genetic disorders in energy balance regulation result in obesity, diabetes and their sequelae. We apply a set of assays to identify metabolic phenotypes in mouse mutants and develop novel mouse models for disturbed body mass regulation or glucose homeostasis. The monitoring of body mass, body composition, indirect calorimetry, activity and drinking/feeding behavior comprise the main tests implemented in the first-line phenotyping pipeline. Tests at GMC are conducted following evaluated standard operating protocols and are now incorporated in the platforms of the International Mouse Phenotyping Consortium (IMPC). Due to the increasing interest as well as enormous international investments in mouse phenotyping there is a strong demand on further refinement of phenotyping assays to provide specific and disease related read outs. In the search for novel biomarkers indicating disturbances in food energy utilization and energy metabolization we introduced two novel technologies now available for mouse phenotyping platforms. Firstly, we developed a novel method for the screening of gene functions in digestion and absorption physiology by high-throughput analysis of feces composition based on Fourier Transformed Infrared (FTIR) spectrometry. Several mouse mutants with impaired digestion efficiency have already been identified using this new technology. Secondly, we set up a test that is based on the analysis of exhaled volatile organic compounds in mouse breath. Hundreds of compounds come into consideration as exhaled VOCs abundant in mouse breath. We hypothesize that this set of compounds will be useful to characterize the metabolic status of a mouse. Therefore, breath gas analysis is considered to be a powerful phenotyping assay for detecting mouse mutants with deviating VOC signature that is related to gene functions in metabolic pathways.

German Mouse Clinic 2 – Behaviour and Psychophysical Stress

Presenting Author: Annemarie Zimprich (1,2)

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The German Mouse Clinic has established a platform, called German Mouse Clinic 2, where genome-environment interactions can be studied in mutant mouse models. Here different challenges, which simulate major environmental challenges to human health, can be applied and several different end points investigated. Stress is one of the major risk factors for the development of neurodegenerative diseases and is highly implicated in many other complex diseases, amongst others psychiatric disorders. To this end we have established an acute and a chronic stress challenge, that reliably induce behavioural changes in mice of both sexes.
Today we are presenting the behavioural read-out of the acute stress challenge, with which we can detect hypo-reactivity and hyper-reactivity in response to acute stress measured in a non-invasive mode in mutant mouse lines. We demonstrate that locomotor and rearing behaviour in the Open Field after an acute stress challenge can be used as reliable, non-invasive indicators of stress reactivity and corticosterone secretion.

The role of cardiac DNA methylation in human dilated cardiomyopathy

Presenting Author: Jan Haas

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In recent years, dilated cardiomyopathies (DCM) have been subject to extensive genetic studies. Despite technological advances such as next-generation sequencing, in many DCM patients a genetic cause could not be unraveled and genetic modifiers only explain a small portion of the phenotypic variability of DCM. In the present study, genome-wide cardiac DNA methylation was examined for the first time in patients with idiopathic DCM and controls. We detected methylation differences in 359 pathways related to heart disease, but also in genes with a yet unknown meaning in DCM or heart failure. The identified genes are associated with different molecular functions and e.g. act as receptors, cell surface molecules or transcriptions factors. We confirmed our results with mass-spectrometric analysis and bisulfite sequencing in a cohort of 30 DCM and 28 control patients. For some candidate genes, aberrant DNA methylation in DCM patients was associated with significant changes in their cardiac mRNA levels, which was further investigated by in vitro promoter analysis. Together with in vivo studies of their orthologous genes in zebrafish, this hints at a relevant role of altered DNA methylation during DCM pathogenesis.

The comparison of beta-globin gene mutations between populations of Mughan region of Azerbaijan and Shiraz region of Iran

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Beta-thalassemia composes more than 90% of all thalassemia types and is endemic for population of some regions of Azerbaijan and Iran. The genetic researches were advanced for revelation people with beta-thalassemia, determination of mutation frequency and types among the Mughan region population of Azerbaijan and Shiraz region of Iran. As methods of ß-thalassemia set by CBC, hemoglobin electrophoresis, study of HbA2 and HbF, genealogical analysis. For the detection of the beta-globin gene mutations, the polymerase chain reaction (PCR) - based technique of amplification refractory mutation system (ARMS) has been used. The method of hemoglobin electrophoresis on acetate-cellulose pellicle and analytical method of hemoglobin isoelectrofocusing in polyacrylamide-ampholine disc with pH 3.5-9.5.
In Mughan region 46 defective beta-globins genes were established, in which 43,48% were IVS-1-110(G-A), 23,91% were codon 8(-AA), 19,57% were IVS-2-1(G-A), 8,69% were IVS-1-5(G-S) and 4,35% were IVS-1-6(T-S). In Shiraz region many types of mutations have installed, among which were the highest frequency of mutation: IVS II-I (G-A), C36-37(-T), IVS I-5(G>C), IVS I-110(G>A), -25bpdel (252-276). In most of the cases (95.8%), the diagnosis was possible by direct mutation analysis and linkage analysis was done to prove mutation analysis. However, in the remaining (4.2%) cases linkage analysis was required. The beta-GG expression full breaks at beta0-phenotype unlike beta+-phenotype. The breach of biosynthesis splicing is the result of IVS-1-110(G-A), IVS-1-5(G-S), IVS-1-6(T-S), IVS-2-1(G-A), C36-37(-T), 25bpdel (252-276) mutations. The nonsense mutation breaching transcription is the result of codon 8(-AA) micro deletion.
The high frequency of blood hereditary breach spreading among the region's population was result of high frequent kinship marriages in these districts. Future work will involve advance of prenatal diagnostics and study of gene polymorphism at families with thalassemia.

Identification and Comparison of BRCA1 and BRCA2 Variants

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Increasing usage of next generation sequencing (NGS) to identify DNA variants specific for certain diseases is an important step towards personalized diagnostics and targeted therapeutics. NGS is usually followed by a series of bioinformatical analysis steps, which include comparisons of newly decoded sequences to a reference, annotation of thereby detected alterations with information from knowledge bases as well as their functional consequences. We here present the analysis of cancer relevant mutations within BRCA1 and BRAC2 genes with CLC Genomics Workbench.

Genomic DNA was extracted from blood cells (11 cancer and 3 control samples). BRAC1 and BRAC2 coding regions were amplified and pooled per individual. Each pool was provided with unique barcodes and sequenced with the Ion Torrent PGM. Using CLC Genomics Workbench, we built an efficient highly automatized re-sequencing workflow for the end-to-end analysis of NGS data. Our in-house algorithms were used to trim and de-multiplex the sequences, map the trimmed reads against the human reference hg19, call the variants and refine the analysis. DNA variants were compared between different samples, findings compared with already publicly reported variants, the list of candidate variants further annotated and their functional consequences accessed. Using the workflow described above, we were able to identify variations (SNPs, MNPs and InDels) present within the BRAC1 and BRCA2 genes with 100% sensitivity. Some of these candidate variants are in accordance with previous findings reported within the COSMIC or HGMD databases others have not been described in the context of cancer before.

The analysis described here can be automated via a workflow tool and runs on high numbers of samples without manual interaction needed. The CLC Genomics Workbench therefore provides a powerful framework for genome-wide analysis of variants in cancer samples, to compare them with knowledge bases and depict their functional consequences.

Identification of functional variants in FOXO3A, a confirmed candidate gene influencing human longevity:

Genetic investigation requires special attention to sequence homology with FOXO3B

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Human longevity is considered a multi-factorial phenotype with a genetic contribution of about 25%. Up to now, variation in only two genes has been identified, which has an effect on longevity in various populations: the apolipoprotein E gene (APOE) and the forkhead box O3A gene (FOXO3A). For FOXO3A the underlying molecular mechanisms still remain to be elucidated, as most of the longevity-associated single nucleotide polymorphisms (SNPs) analyzed are located in intronic regions and the “functionally relevant” SNP has yet to be identified. To explore potential unknown genetic variants both Next Generation and Sanger sequencing are being applied. Functional effects of all identified rare and common SNPs are evaluated by in silico analyses. Variants of interest are genotyped in our extensive German longevity sample (~1400 long-lived cases and 1100 younger controls). In our study we could recently demonstrate that the genetic investigation of FOXO3A is greatly hampered by the fact that the exonic regions have 99% sequence homology with the FOXO3B pseudogene. If unaccounted for, this high degree of homology can cause serious genotyping or sequencing errors. Here, we present an experimental set-up that allows reliable data generation for the highly homologous regions and that can be used for evaluation of assay specificity. Using this design, we could exemplarily show FOXO3A-specific results for two SNPs that are significantly associated with longevity in our German longevity sample (Peach = 0.0008). Because both SNPs are located in the 3’UTR of FOXO3A, they could be of functional relevance for the longevity phenotype. In the following, various in vitro and ex vivo assays will be applied for the functional characterization (e.g. gene expression profiles, splicing events) of associated variants. Subsequently, the functional assessment of the FOXO3A gene region could serve as the basis for future treatment strategies to achieve healthy ageing in the elderly.

A novel more practical approach of genome lift over: Save time in imputing your study data

Presenting Author: Mathias M. Gorski

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Background: Imputation of genotypes into a reference panel is a statistical approach that can be used for genome wide association analysis to evaluate association at genetic markers that are not directly genotyped. The lately introduced pre-phasing method [Howie, et al. 2012] separates between phasing genotypes to haplotypes in the first step and imputing these resulting haplotypes with a reference panel to get imputed genotypes in a second step. For imputation it is important that both haplotypes and reference panel are annotated on the same build. Translating genetic data (genotypes, haplotypes or imputed data) from one genome build to another is called lifting. It is the aim of our investigations, to test if lifting genotypes before phasing (prephasing approach) is preferable to lifting haplotypes after phasing (postphasing approach) by examination of imputation quality, association signals and computational costs.

Methods and Results: Both approaches have been compared in our genome wide association study [Wichmann, et al. 2005], consisting of 1644 unrelated individuals, which has been imputed to raise the number of non-monomorphic SNPs genotyped on an Affymetrix 500K SNP array from nearly 500.000 to more than 30 Million. In our approach we phase the genotyped sample using Mach 1.0.16.b [Li, et al. 2010] and impute the resulting haplotypes with minimac 4.4.3 [Howie, et al. 2012], using the GIANT Phase I v3 ALL Reference Panel of the 1000 Genomes Project [2010]. For association analysis a simulated phenotype has been used. The general advantage of our method is the optimal fit of imputed genotypes to the reference panel in respect of SNPnames and positions. The postphasing approach may furthermore require the time intensive phasing process once, so that existing haplotypes may be re-impute faster, as new reference panels are released. When time consumption and computational costs are not a limiting factor, it might be preferable to use the prehasing approach, in which phasing has to be done multiple times for each new released build. This approach leads to more accurate phasing and thus also to more accurately imputed genotypes.

Conclusion: Our approaches may help analysts to lift study data in a fast and easy way. Depending on the available time and computational power, analysts can weigh between gain in accuracy (prephasing approach) and the flexibility to re-impute haplotypes fast, as new reference panels are released (postphasing approach).

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Founder effect analysis of disease haplotypes in DFNB23/USH1F linked Pakistani families

Presenting Author: Dr Riffat Mehboob

Riffat Mehboob (1) and Sheikh Riazuddin(2)

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Usher syndromes are a group of autosomal recessive disorders characterized by moderate to profound sensorineural hearing loss and progressive visual loss from retinitis pigmentosa. Clinically they are classified into three types on the basis of phenotypes. Within each clinical group molecular heterogeneity exists and people with indistinguishable phenotypes have mutations in different genes. Protocadherin-15 (PCDH15) is one of the five genes identified as being mutated in Usher 1 syndrome and defines Usher syndrome type 1F (USH1F). Mutation in this gene also cause nonsyndromic deafness DFNB23.

A total of 25 families were collected in which pattern of inheritance was autosomal recessive and were screened for locus DFNB23 by using fluorescently labeled markers D10S2529, D10S546, and D10S2522. Three families were found to be linked with DFNB23. Haplotypes of these families were compared with 12 previously linked families obtained from CEMB repository. Seven families divided into two groups shared same haplotypes while in other eight families, no correlation was found between the haplotypes.

Variability of haplotypes among families indicate presence of different type of mutations and families with same haplotypes may have same founder. These results will lead to better understanding of hearing impairment caused by mutations in PCDH15 and will help in identification of carriers and genetic counselling.

Multiple Novel Loci Highlighting Metabolic Control of Urate Production and Excretion are Associated with Gout

Presenting Author: Alexander Teumer

Alexander Teumer (1), Anna Köttgen (2,3), Eva Albrecht (4), Veronique Vitart (5), Jan Krumsiek (6), Marina Ciullo (7), Caroline Fox (8,9,10), Mark Caulfield (11), Murielle Bochud (12), Christian Gieger (4), on behalf of the GUGC Consortium (13)

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Uric acid is a final breakdown product of purine oxidation in humans and present in the blood as urate. Hyperuricemia, elevated levels of serum urate, can cause gout, the most prevalent inflammatory arthritis in developed countries. Furthermore, increased levels of serum urate are associated with obesity, blood pressure and insulin resistance metabolic syndrome, type 2 diabetes and cardiovascular disease. The heritability of serum urate concentrations is estimated at 50%-70%. Eleven loci identified by previous genome-wide association studies (GWAS) only explain 5-6% of serum urate variance, suggesting that additional loci remain to be identified.
We performed a meta-analysis of GWAS on serum urate concentrations among 48 studies with more than 110,000 participants of European ancestry, and on gout among >70,000 individuals (3,151 cases) in the Global Urate Genetics Consortium (GUGC). Secondary analyses included, among others, stratification by sex, pathway analyses, and look-ups of the associated loci in individuals of other ancestries as well as with urate-correlated traits. Replication was performed in 32,813 independent samples.
Altogether, we identified and replicated 26 genome-wide significant SNPs associated with serum urate concentrations, including 18 new loci. Nominal association with gout was found for 17 of the SNPs, with consistent directions of effect. No sex-specific effects besides the known for SLC2A9 and ABCG2 were found. Network analyses identified two additional novel genome-wide significant loci. Effect sizes were similar among individuals of Indian ancestry, African Americans and Japanese individuals. An effect-size weighted genetic urate score was significantly associated only with plasma C-reactive protein concentrations.
The genes implicated by our screen highlight the importance of metabolic control of urate production and excretion, and may have implications for the treatment and prevention of gout.

Understanding and building robust clinical assays to analyze microRNAs in biofluids

Presenting Author: Roman Kurek

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microRNAs (miRNAs) constitute a class of small RNAs (typically 19-23 nt) that function as post-transcriptional regulators of gene expression. Current estimates indicate that more than one third of the cellular transcriptome is regulated by miRNAs, although they are relatively few in number (less than 2000 human miRNAs).
The high relative stability of miRNAs in common clinical source materials (FFPE blocks, plasma, serum, urine, saliva, etc.) and the ability of microRNA expression profiles to accurately classify discrete tissue types and specific disease states have positioned microRNAs as promising new biomarkers for diagnostic application. Furthermore miRNAs have been shown to be actively exported from tissues into the circulation with the development of pathology.
The main challenge in detecting miRNA is their small size, no longer than one standard PCR primer. Furthermore, many miRNAs belong to families with only a few mismatches between members, and the G/C content varies greatly. To overcome these obstacles and facilitate discovery and clinical development of miRNA-based biomarkers in biofluids, we developed an LNA™-based miRNA PCR platform with unparalleled sensitivity and robustness. The platform uses a single RT reaction per sample combined with two target specific, LNA™ enhanced PCR primers per miRNA assay to profile human miRNAs and thus allows high-throughput profiling of miRNAs from important clinical sources, with high specificity and sensitivity, without the need for pre-amplification.
Using the LNA™ PCR system, we have profiled thousands of biofluid samples. An extensive QC system has been implemented in order to secure technical excellence and reveal any unwanted bias in the dataset. We will present our approaches to sample handling, qPCR technology, data normalization and studies of pre-analytical variables such as hemolysis.

National Genome Research Network

Poster Presentation Abstracts

Symposium V

Functional Genomics

Novel recombinant AAV gene delivery systems for secreted proteins

Presenting Author: Ann-Marie Heinonen

Ann-Marie Heinonen (1,4), Mahbubur Rahman (2), Anja Groß (1), Peter H. Seeburg (1), Stefan Wölfl (3,4), Markus Schwaninger (2,4), Rolf Sprengel (1,4)

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Here, we designed novel recombinant adeno-associated viral (rAAV) vectors to facilitate long term hormone signaling in the mouse brain. For efficient coexpression of multiple viral transduced genes including secreted and reporter proteins the 2A peptide was used as linker among genes. To establish the system we used bone morphogenic proteins (BMPs) that are multifunctional growth hormones of the transforming growth factor family ß (TGFß) of secreted proteins. The BMPs signal through serine/threonine kinase receptors thereby regulating many key steps to control cell fate, proliferation and differentiation during development. The BMPs have been implicated as critical regulators in the developing central nervous system (CNS). However, the widespread and persistent expression of BMPs and BMP receptors in mature neural tissue suggests that they also play an important role in the adult CNS. One potential role of BMPs in the adult CNS is their involvement in functional recovery from injury.

Our data show that secretory proteins linked to a reporter protein can be efficiently coexpressed via the 2A peptide using rAAV-mediated gene transfer in vitro and in vivo. The biological activity of 2A-released proteins was demonstrated by the stimulation of down-stream signaling cascades. Expression of BMP7 after rAAV transduction has protective properties in experimental models of cerebral ischemia.

Immediate early gene expressing in the GluA1-deficient mouse brain in learning

Presenting Author: Boyi Yang

Doruk Okbay, Boyi Yang, Rolf Sprengel

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AMPA receptors in the CNS are glutamate-activated ion channels that are important in the transfer of excitatory signals and that are critical involved in spatial working memory. We investigated the neuronal activity in the hippocampus of Gria1-/- and Gria1R/R mice (both of which show no functional GluA1 based AMPA receptors in the postsynaptic membrane) and used the immediate early gene c-fos as molecular marker for neuronal activity. After analyzing the c-fos expression in novel environments in the wild-type mice, we tested the behavior of Gria1-/- and Gria1R/R mice in a novel environment. Then we used novel spatial elements in the now familiar environment to induce spatial learning in the Gria1-/-, Gria1R/R and wild-type mice, and compared the hippocampal c-fos expression. In the third part of this study we applied the fear-conditioning process to Gria1-/- and wild-type mice to analyze the neuronal activity pattern in the amygdala during the acquisition of conditioned fear. Our findings support the already established idea that the GluA1-deficient mice have an impaired short-term memory, while the long-term spatial memory is more effective. The overexpression of c-fos in the dentate gyrus (DG) of the mutant animals after experience a novel object suggests the involvement of the DG in the acquisition of long-term memory. During fear-conditioning the Gria1-/- KO animals showed a considerable lack of fear-expression, which could mean the GluA1 subunit is also of importance for emotional short-term memory. The difference in the c-fos expression pattern of amygdala in both groups of mice could be observed as c-fos hyperexpression in the central amygdaloid nuclei of the Gria1-/- KO animals. Thus, this structure is directly linked to the emotional-memory phenotype of the Gria1-/- mice.

SCN2A mutation associated with BFNIS leads to hyperexcitability in the mouse brain

Presenting Author: Birgit Engeland

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Voltage-gated sodium channels are essential to initiate action potentials in brain neurons. Mutations of the SCN2A gene encoding NaV1.2 are associated with benign familial neonatal infantile seizures (BFNIS) or severe epileptic encephalopathy. Recently we identified a missense mutation in SCN2A (A263V). The patient carrying this mutation presented with neonatal-onset, pharmacoresistant seizures and later onset variable episodes of ataxia, myoclonia, headache and back pain after 18 months of age. Electrophysiological studies in a heterologous expression system indicated that this SCN2A mutation leads to an increase of the persistent sodium current revealing a pronounced NaV1.2 gain-of-function effect (Liao et al. Neurology 2010;75:1454-8). We generated SCN2A knock-in mice carrying the human point mutation. Homozygous and heterozygous knock-in mice are smaller and lighter than their littermates. Seizures are observed in homozygous mice starting at three weeks. Behavioural studies in adult mice revealed hyperactivity and cognitive impairment, which is more pronounced in homozygous animals.
Current-clamp experiments in acute brain slices indicated an increased frequency in action potential firing in hippocampal CA1 pyramidal neurons in heterozygous SCN2A mutant mice compared to their wild-type littermates. These results suggest that an increased excitability of pyramidal neurons caused by a gain-of function of the NaV1.2 channel underlies benign familial neonatal infantile seizures. Other symptoms such as ataxia, myoclonus or pain might be caused by similarly increased excitability in unmyelinated nerve fibers in which SCN2A is expressed later in development than in myelinated pyramidal neurons (Liao et al. Neurology 2010;75:1454-8).

A Large Turkish Parkinson Pedigree with alpha-Synuclein Duplication:

Blood Expression Biomarker Profile for Predictive Diagnostics

Presenting Author: Georg Auburger

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Parkinson's disease (PD) is a neurodegenerative disorder, affecting 2% of the population at old age. While most cases are sporadic, monogenic variants are found with early-onset. Alpha-synuclein (SNCA) was the first Parkinson's disease gene, identified in autosomal dominant pedigrees. Missense mutations and increased gene dosage cause monogenic fully penetrant PD, while promoter/3'UTR polymorphisms enhance risk and constitute prominent risk factors in sporadic PD GWAS. Alpha-synuclein aggregates in degenerating neurons, forming cytoplasmic "Lewy bodies", the diagnostic hallmark upon autopsy.

We now identified a Parkinson pedigree cosegregating alpha-synuclein gene duplication, with 2 patients and 12 presymptomatic mutation carriers. To study alpha-synuclein gain-of-function effects in whole blood, the 1.5-fold increase in transcript and monomeric protein levels were documented. Oligomers of alpha-synuclein protein and high molecular weight aggregates were not detected in blood. Although the SNCA transcript levels showed high variance and their correlation with genotype did not attain statistical significance, mutation effects on the expression of several downstream genes were detectable, partially in significant association or statistical trends. Receiver operating characteristics of the combined mRNA biomarker profile showed high predictive value.

Its usefulness for subgroup diagnostics in Parkinsonism and for risk prediction of old-age sporadic PD remains to be tested.

Cav1.3 channels stabilize D2-autoreceptor function via NCS-1 in substantia nigra dopamine neurons after in vivo cocaine.

Presenting Author: Elena Dragicevic

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Substantia nigra (SN DA) dopamine midbrain neurons are important for voluntary movement control as well as for habit formation, and are particularly prone to degeneration in Parkinson's disease (PD) and its animal models. Systemic L-type Ca2+ channel (LTCC) blockers protect SN DA neurons in PD and its mouse models, but the precise physiological roles of LTCCs in SN DA neurons remain unclear. Here, we identify a novel role for Cav1.3 LTCCs in mouse SN DA neurons. By analyzing respective juvenile and adult mouse models, we show that the activity of LTTCs (in particular Cav1.3) is crucial for age-dependent stabilization of somatodendritic dopamine-D2-autoreceptor (D2-AR) function. D2-ARs, coupled to GIRK2 channels, modulate spontaneous electrical activity and subsequent dopamine release of SN DA neurons in a negative feedback loop. D2-receptors are established therapeutic targets in PD, and sensitization processes due to chronic agonist treatment have been implicated to negative side effects. Differences in functional expression of D2-ARs have also been associated with novelty seeking and vulnerability to addiction. We found that therapeutically relevant pulsatile high-dopaminergic states (single i.p. dose of 15mg/kg cocaine in vivo) induced an age-dependent stabilization and sensitization of D2-AR responses of SN DA neurons in vitro (brain slice perforated patch clamp recordings). Mechanistically, this process involves isradipine-sensitive (300nM) Cav1.3 activity, Ca2+, and interaction of the neuronal calcium sensor 1 (NCS-1, showing elevated mRNA-levels after cocaine in juvenile SN DA) with D2-ARs. The AMPAR/NMDAR ratio was not altered in vitro in juvenile SN DA neurons after in vivo cocaine. Moreover, we detected an orchestrated transcriptional dysregulation of NCS-1, D2-AR, and GIRK2 in surviving human SN DA neurons from PD patients, indicating that this novel Cav1.3/NCS-1 dependent D2-AR signaling is also operative in humans and contributes to PD pathophysiology.

Validating P2RX7 as a susceptibility marker for mood disorders using humanized mouse models

Presenting Author: Jan M. Deussing

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The P2RX7 gene encodes the purinergic P2X7 receptor (P2X7R), which is a member of the P2X family of ATP-gated ion channels. Particularly the non-synonymous single nucleotide polymorphism (SNP) rs2230912, which leads to a substitution of glutamine (Gln) by arginine (Arg) at codon 460 (Gln460Arg), has been tested for its association with mood disorders. While several studies reported an association of this SNP with mood disorders, other studies did not detect significant associations. To investigate its functional significance in vivo we generated knock-in mice expressing human wild-type P2X7R (hP2X7R-WT) and hP2X7R-Gln460Arg, respectively. The substitution of mouse P2X7R and correct expression of the human P2RX7 variants in the brain of mutant mice was confirmed by in situ hybridization using a human-specific probe. Both humanized P2X7R mouse lines, either homozygous for the wild-type hP2X7R-Gln460 or for the mutant hP2X7R-Arg460 express a fully functional P2X7R as indicated by their capability to induce the release of IL-1ß from peritoneal macrophages. Behavioral phenotyping of humanized mice revealed an attenuated acoustic startle response and a reduced quality of sleep in heterozygous hP2X7R mice. These phenotypes might resemble a pre-symptomatic disease stage or indicate an increased vulnerability of heterozygote hP2X7R mice to develop symptoms of mood disorders. Since stress in combination with a genetic predisposition can act as a trigger for processes that result in an increased risk of developing mood disorders, we subjected hP2RX7 mice to three weeks of chronic social defeat stress. Interestingly, mice heterozygous for both variants showed an altered stress response compared to their homozygous littermates supporting a heterozygous disadvantage model. Taken together, these humanized mouse lines are significantly contributing to the functional validation of human association data related to P2RX7.

Assessing CRH-mediated anxiogenic and anxiolytic effects using brain region- and neurotransmitter type-specific overexpressing mice

Presenting Author: Jan M. Deussing

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The corticotropin-releasing hormone (CRH) and its type 1 high-affinity receptor (CRHR1) orchestrate the neuroendocrine and behavioral adaptation to stress. Dysregulated and/or hyperactive CRH/CRHR1-circuits have been implicated in the pathophysiology of stress-related disorders such as anxiety and depression. So far little was known about the specific CRH-controlled circuits which modulate anxiety and depression-like behavior. We could recently provide evidence that anxiety-related behavior is generated by an imbalance between CRHR1-controlled anxiogenic glutamatergic and anxiolytic dopaminergic systems. However, the identity of CRH-releasing neurons and sites of CRH action have not been fully established yet. Thus, we applied conditional strategies to generate transgenic mouse models overexpressing CRH in a region- or neurotransmitter type-specific fashion. We could show that CRH overexpression in forebrain principal neurons (CRH-COE-FB) enhances anxiety-related behavior whereas overexpression specifically in GABAergic neurons (CRH-COE-GABA) produces the opposite effect. Thus, our results further support that CRH can modulate anxiety-related behavior in opposite directions via different neurotransmitter circuits. With respect to clinical findings and in order to fully understand the effects of CRH hyperdrive in the context of anxiety and depression, the generation of mice overexpressing CRH under its endogenous promoter represents a matter of particular importance. By breeding the recently generated CRH-ires-Cre mice to our conditional mouse mutants we obtained a mouse model of CRH hyperdrive (CRH-COE-Crh), which fully recapitulates the endogenous CRH expression pattern. Future behavioral and molecular analysis of CRH-COE-Crh mice might provide essential and still missing insights into the role of CRH in mood and anxiety-disorders.

Reward processing as a schizophrenia intermediate phenotype: reliability, genetic risk, and effects of genome-wide supported risk variants

Presenting Author: Oliver Grimm

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Schizophrenia is characterized by positive symptoms like delusionsor negative symptoms like anhedonia. As a pathophysiologic mechanism, dysregulated presynaptic dopaminergic signaling in the striatum is well established. As a functional correlate, previous fMRI studies point to a hypoactivation of the striatum during reward anticipation in patients. However, no study so far has examined disease-related heritability of this intermediate phenotype. Therefore, our study was aimed at testing whether striatal activation is a reliable fMRI measure, whether striatal hypoactivation represents is related to genetic risk by studying first-degree relatives of patients, and whether genome-wide significant SNPs are associated with this intermediate phenotype.

MRI Scanning was done with a previously established reward-anticipation task. Study I: 53 healthy first-degree relatives were compared with 80 matched controls. Study II: reliability study in 25 volunteers. Study III: Analysis of genetic association in a sample of n=320 healthy volunteers.

SNPs for association analysis were derived from recent consensus reviews, genotyped on DNA-chips and imputed with IMPUTE2. Association analysis was done with mean extracted fMRI beta value from a striatal mask.

In Study I, first-degree relatives showed a significant hypoactivation of the striatum during reward anticipation. In Study II, the significant cluster from Study I showed a high reliability. In study III, rs2239547 was correlated with significant hypoactivation during reward anticipation. Our three studies indicate that striatal hypoactivation during reward anticipation is a reliable intermediate phenotype for schizophrenia. Additionally, it is associated with hypoactivation in rs2239547, a SNP that shows genome-wide support for association with schizophrenia. Future studies should clarify the neurobiologic function of rs2239547 and should evaluate disease specificity of the striatal hypoactivation during reward anticipation.

The risk variant in ODZ4 for Bipolar Disorder impacts on amygdala activation during reward processing

Presenting Author: Angela Heinrich

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Background: The largest GWAS analysis of bipolar disorder (BD) recently identified a new risk variant in ODZ4 (rs12576775). We investigated whether this gene influences altered brain functions described in BD such as increased BOLD responses in the amygdala in response to reward (Linke, King et al. 2012) and dysfunctions in a ventral-limbic brain network during emotional stimuli (Wessa and Linke 2009).
Methods: With an imaging genetics approach we analyzed the impact of the ODZ4 rs12576775 (G / A alleles) on processing emotion and reward in the amygdala and the striatum in 485 healthy adolescents from the Imaging Genetics study (Schumann, Loth et al. 2010). Three-hundred-and-forty-seven subjects were homozygous A-allele carriers, 126 were heterozygous, and 12 were homozygous G-allele carriers (Hardy-Weinberg Equilibrium: p = 1.00). For the analysis of processing emotion and reward we utilized a Face-task (Grosbras and Paus 2006) and a modification of the monetary incentive delay (MID) task (Knutson, Adams et al. 2001). Scanning was performed with 3T whole body MRI systems and the data were analyzed with SPM8.
Results:Carriers of the risk variant in ODZ4 rs12576775 showed a significantly increased BOLD response in the amygdala during both reward sensitivity (win versus no win in the feedback phase) and reward expectation (win versus missed time criterion in the feedback phase) components of the MID task (pFWEcorrected = 0.05, see Figure 1). No significant differences between the two groups were found in the amygdala and the striatum during the Face-task.
Discussion:We could show that the ODZ4 rs12576775 risk variant contributes to an increased BOLD response in the amygdala in a reward sensitivity and reward expectation paradigm, with carriers of the risk allele more strongly involving the amygdala in reward processing. The absence of significant group differences in emotion processing (Face task) suggests that the risk variant does not affect this capacity.

Phactr1 determines the severity of calcification in murine embryonic stem cells

Presenting Author: Redouane Aherrahrou

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Introduction:
Calcification of vessels is strongly associated with atherosclerosis and leads to coronary artery disease (CAD) and myocardial infarction (MI). Until now genome wide association studies (GWAS) revealed several genes that are associated with and contribute to CAD/MI as well as coronary artery calcification (CAC) but the underlying mechanisms are still unknown. Phactr1 is among these risk genes which encodes for the phosphatase and actin regulator 1.
Aim of study:
The aim of this study was to functionally test whether Phactr1 regulates calcification in-vitro using murine embryonic stem cells (mESC). Up and down regulation of Phactr1 was carried out using ShRNA technology and cDNA-Phactr1 plasmid in mESC.
Material and Methods:
Induction of calcification in mESC was performed by treatment with retinoic acid followed by the addition of calcifying medium (StemXVivo™ Human/Mouse Osteogenic, R&D). Calcium phosphate deposits were confirmed using Alizarin red S staining and quantified using a commercial Ca2+Kit (Ca, Randox Laboratories LTD).
Results:
Using RT-PCR the gene expression of Phactr1 was found to correlate with increased calcification in calcifying mESC. Down regulation of Phactr1 showed significant decrease in calcification. Furthermore downregulation of Phactr1 gene expression and osteogenic marker osteopontin confirm this finding at molecular level. Vice versa, overexpressing Phactr1 in calcifying mESC enhanced mineralization.
Conclusion:
Our data show for the first time the implication of Phactr1 in calcification in-vitro and may reflect the role of Phactr1 in arterial calcification.

Functional investigation of the role of the CAD-risk gene ZC3HC1 on atherosclerosis

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Atherosclerotic lesion of the coronary vessels is associated with coronary artery disease (CAD). ZC3HC1, the gene encoding for NIPA (Nuclear Interaction Partner of Anaplastic Lymphoma Kinase), a protein that plays a role in cell cycle, has shown a strong association with CAD in genome-wide association studies (GWAS).
We aim to functionally investigate the role of NIPA on atherosclerosis in a knockout (KO) mouse model, especially, the effect of the complete deficiency of NIPA on changes in the overall phenotype and the formation of atherosclerotic lesions.
Heterozygous ZC3HC1-KO mice were obtained from the University of California, Davis (UC Davis), and intercrossed, to generate homozygous ZC3HC1-KO mice. In parallel, backcrossing to the proatherogenic ApoE-background is ongoing to generate ZC3HC1-ApoE double KO (dKO) mice.
ZC3HC1-KO mice were investigated to gain general insights on birth rate, body weight and behaviour. KO mice were born smaller and with a birth rate of nearly 12%, about 50% lower than the expected 25% mendelian ratio. Weekly weight controls for more than 4 months showed that the KO animals remain alive but smaller over this period of growth compared to wild type animals.
The formation of atherosclerosis depends on different cell types, i.e. immune cells (IC), smooth muscle cells (SMC) and endothelial cells (EC), to migrate and proliferate. The influence of ZC3HC1 on these cell functions will be assessed in-vitro, using isolated primary IC, SMC and EC, and in-vivo, using neointima formation after carotis ligation. In addition, lipid profiles, sectioning and determination of atherosclerotic lesions development and plaque stability will be investigated in ZC3HC1-ApoE dKO mice vs. ApoE-KO mice in more detail after feeding a high fat diet.
Our data suggest a critical role of NIPA on viability and growth. However, further studies are required to test the effect of this protein on atherosclerosis and inflammation using various in-vitro and in-vivo analyses.

Loss of macrophage infiltration and B-cell function in MRas- knock-out mouse

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Recently, we identified by genome-wide association study MRas gene locus on human chromosome 3q22 to be associated with coronary disease (rs9818870; p=7.44x10-13). M-Ras is a member of the Ras superfamily of small GTPases, which act as molecular switches in diverse cellular functions and thereby regulate a variety of biological processes. M-Ras has been implicated in the regulation of TNFa-stimulated LFA-1 activation and integrin-mediated leukocyte adhesion downstream of various inflammatory cytokines.
To further study the pathomechanism underlying the the association between MRas and CAD in a knockout mouse model.
We crossbred the MRas-KO mice onto the ApoE-KO background and fed these mice with western diet. In parallel, we performed adhesion and migration assays with macrophages, monocytes, B- and T-cells to study the influence of MRas on adhesion and migration as a potential pathomechanism of atherosclerosis.
The MRas/ApoE-KO mouse showed no difference in plaque size at the aortic root in comparison to the ApoE-KO mouse after 10 weeks of high fat diet, but there are significant less macrophages in the plaque (p=0,002). In addition, the ratio between macrophage and collagen in the plaque at the aortic root is significant higher in the MRas/ApoE-KO than in the ApoE-KO under high fat diet (p=0,002).
Furthermore, we observed a 15% reduction of CD19+ B-cells in lymphnodes in MRas-KO mice ($p<0.05$), but not in bone marrow, spleen and blood. Moreover, we noticed a 20% reduction of leukocyte adhesion in in vitro assays with an endothelial cell line ($p<0.05$). Again, especially B-cell adhesion is reduced in MRas-KO mice in comparison to WT mice. In vitro migration assays show also a reduced migration of B-cells towards the chemokine CxCl12 ($p<0.01$).
Our data suggests that MRas-KO has no effect on plaque formation at the aortic root but on the plaque stability by less macrophage infiltration. This could be linked to the reduction of adhesion and migration by B-cells.

Utilizing Patient Specific Induced Pluripotent Stem Cell Derivatives to Functionally Characterize Dilated Cardiomyopathy Risk Alleles

Presenting Author: Maya Fürstenau-Sharp

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Dilated cardiomyopathy (DCM) is a common heart disease affecting the myocardium. The major characteristic of DCM is systolic dysfunction resulting in ventricular dilation as well as impaired function and eventually heart failure. While about 35% of patients have familial forms of the disease, it has been presumed that genetic factors also contribute to idiopathic cases of DCM. In order to diagnose DCM in its familial and idiopathic form to initiate treatment prior to its onset, the identification of risk alleles is crucial.

Through a genome-wide candidate gene study, we recently identified several candidate risk genes for idiopathic DCM. Individuals homozygous for the major allele of one the identified candidate genes have a 30% increased risk to develop DCM while those homozygous for the minor allele are at a 40% decreased DCM risk. In addition, allele specific gene expression studies in heterozygous human cardiomyocytes have demonstrated that the major allele is expressed 10 fold less than the minor allele. These results suggest that this particular candidate gene might be a protective factor in cardiac muscle cells.

To functionally characterize this gene, we decided to utilize human induced pluripotent stem (iPS) cell derived cardiomyocytes to model DCM in vitro. This approach enables us to create a more relevant, disease-specific cellular model.

We already generated iPS cell derived cardiomyocytes from an individual homozygous for the protective allele. These cardiomyocytes carry a lentiviral construct expressing a ventricular specific promotor fused to an antibiotic resistance gene and a DOX inducible risk gene specific shRNA. This construct enables us to knock down the respective risk gene in pure ventricular cardiomyocytes.

To assess the function of the DCM risk genes of interest, we will compare ventricular cardiomyocyte morphology, biochemistry and function pre- and post risk gene knock down.

MetaXpress: Population-based transcriptome analyses of hypertension parameters

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Hypertension is associated with an increased risk of cardiovascular events and it is influenced by multiple biological pathways and environmental stimuli. Meta-analyses in 200,000 individuals identified various loci showing associations with blood pressure and cardiovascular disease. To date, the correlation between the transcriptome and hypertension has not been studied on a population based level.

The aims of this study were to analyze associations between gene expression and systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) by applying standardized protocols. For this we formed the MetaXpress consortium and investigated gene expression data based on the German cohorts GHS, KORA F4 and SHIP-TREND.

In 3,358 individuals gene expression levels were measured in monocytes (n=1,374) and whole blood cells (n=1,984) using Illumina HumanHT-12 BeadChip arrays. Associations to SBP, DBP and PP were computed by applying linear regression models adjusted for sex, age, RNA quality, plate layout and sample storage time. Functional analyses of significantly differential expressed genes (FDR<=0.05) were conducted using IPA (Ingenuity Pathway Analysis).

In monocytes/whole blood 263/232 genes were associated with SBP, 124/1136 with DBP and 143/0 with PP. Among the strongest differentially expressed genes we found histidine decarboxylase (HDC), endothelial transcription factor GATA-2 (GATA2) and ABC transporter member 1 of subfamily G (ABCG1). IPA-analyses identified significant over-representations of genes within the inflammatory IL-4-, IL-6-, IL-10- and p38-MAPK-signaling pathways. Further analyses indicated a relation between activation of platelet derived growth factor beta (PDGFB) and high blood pressure (SBP and PP). The first results of our gene expression analyses for blood pressure related phenotypes within the MetaXpress consortium suggest an involvement of inflammatory and immune responsive pathways in hypertension.

Differential gene expression and functional analyses in male patients with early-onset of myocardial-infarction

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Introduction:
Myocardial infarction (MI) is one of the main causes of mortality and is usually a disease of the middle-aged and elderly. MI in young adults is a rare phenomenon and the molecular mechanisms of the genetic predisposition of MI at young age remain unclear. Transcriptome analysis is a promising tool to explore disease-related genes and biological pathways. The aims of this study were to identify differentially expressed genes in young male MI individuals and to characterize these genes by functional analyses.
Methods:
In 94 male patients (age at MI <= 45) and 173 healthy controls gene expression levels in peripheral blood mononuclear cells were measured using Affymetrix GeneChip exon arrays. Differential gene expression was computed by applying linear regression models adjusted for the risk factors sex, age, BMI, hypertension, smoking status, diabetes and LDL/HDL ratio and the first four components of the principal component analysis. Functional analyses of significantly differential expressed genes (FDR<=0.05) were conducted using IPA (Ingenuity Pathway Analysis).
Results:
247 genes were significantly associated with MI. Among the strongest differentially expressed genes we found the G protein-coupled receptors GPR15 (G protein-coupled receptor 15) and P2RY2 (ATP receptor) as well as the genes PGS1 (phosphatidylglycerophosphate synthase 1) and ABCA1 (ATP-binding cassette transporter A1). IPA-analyses identified significant over-representations of genes within the phospholipid pathways phosphatidylglycerol biosynthesis II and CDP-diacylglycerol biosynthesis I and in the inflammatory IL-10-signaling pathway. Transcription factor analyses indicated an inhibition of MAPK1 (mitogen-activated protein kinase 1).
Conclusion:
The results of the differential expression analyses in young male MI patients suggest changes in phospholipid metabolism and inflammatory pathways.

Diverse functional effects of genetic deletion of CAD risk genes in mice: CardioGENE KO-Project

Presenting Author: Zouhair Aherrahrou

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Coronary artery disease (CAD) is a complex disease. Recently, 46 genomic loci have been identified to be associated with CAD using genome-wide association studies (GWAS). However the functional role of the identified CAD risk genes remain completely unknown.
We aim to functionally investigate the role of seven CAD risk genes in mouse models.
Firstly, we generated the KO mice for each gene and studied the effects of global gene deletion on growth, fertility, viability and behavior in these mice. Secondly, additional cardiovascular phenotyping screens including in-vivo vascular injury models as well as in-vitro migration and proliferation assays were applied. Thirdly, KO mice were backcrossed into a proatherogenic background (ApoE- or Ldlr-KO-mice) to study atherosclerosis.
Mras deficient mice display metabolic dysfunction and an impaired migration of B-cells. ADAMTS-7 plays a key role in the extracellular degradation leading to enhanced neointima formation after vascular injury. Gucy1a3-KO mice presented with loss of NO mediated inhibition of platelet aggregation. Zc3hc1-KO-mice lacking the encoding NIPA protein, involved in cell cycle, display abnormal viability and markedly decreased body weight. Cyp17a1 encodes for a member of the cytochrome P450 superfamily of enzymes, which are involved in cholesterol synthesis, drug and steroid metabolisms. Cyp17a1-KO-mice have abnormal steroid metabolism and display congenital adrenal hyperplasia. Cxcl12 and Ppap2b deficiency is lethal. Atherosclerotic lesion studies after feeding a high fat diet are still in progress.

Deletion of each of the CAD risk genes in mice leads to high variability of phenotypes. Traditional risk factors such as hypercholesterolemia or hypertension are only involved in some mice deficient for CAD risk genes. These results suggest a diverse array of cellular functions exerted by newly identified CAD risk genes, which reflects the complex mechanisms leading to CAD.

Molecular role of microRNA-582, a novel microRNAs differentially regulated in dilated cardiomyopathy

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MicroRNAs are a class of short non-coding RNAs, which are involved in post-transcriptional regulation. We have previously identified a differentially expressed microRNA, mmu-mir-582, in a murine model of dilated cardiomyopathy (DCM). The aim of this study was to further clarify the potential role of this microRNA.

A bioinformatics screen of potential target mRNAs for mmu-miR-582 resulted in a number of binding sites and native 3'-UTRs which were screened for functional binding motifs with a dual luciferase reporter system. Either mature microRNA alone or pre-miRNA-based expression constructs showed differential abilities to suppress luciferase activity when using positive control vector. From all tested mmu-miR-582-5p targets, Art1 and Rcan3, and mmu-miR-582-3p targets Pcdh19 binding sites showed strongest effects, respectively ($p<0,09$). When testing larger parts or complete 3'-UTRs we found significant regulation of Csrp3 (MLP), Pde4d (the microRNA-582 host gene), Vegfb and Prkd1 (all $p<0,05$). To further assess the role of mir-582, its expression levels were also measured in an in vitro model of cardiac hypertrophy, namely neonatal rat ventricular cardiomyocytes subjected to cyclic stretch for 24 hours or phenylephrine treatment. Interestingly, only cyclic stretch resulted in a 1,5-fold reduced expression of miR-582-5p while agonist treatment did not lead to any expression changes.

In conclusion, we have identified murine mir-582 as differentially regulated in a murine knockout model of DCM, implicating a possible role of this microRNA in the etiology of cardiomyopathies. Furthermore, mechanical stretch lowered while PE treatment did not alter mir-582 expression. Collectively, these data imply a potential role in the stretch sensing cellular machinery. First results from luciferase reporter experiments have indicated a number of potential target mRNAs, including the miRNA-582 host gene Pde4d, thereby suggesting a possible feedback regulation.

Myoscape (Muscle enriched Calcium-Channel associated protein), a novel modulator of L-type Calcium Channel dependent calcium currents and contractile function in vivo and in vitro

Presenting Author: Matthias Eden

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Alterations in cardiac calcium fluxes, e.g. the EC coupling machinery have been shown to play a major role in the progression of contractile dysfunction. The voltage dependent L-type Calcium Channel is one key regulator of cellular calcium influx and a major component of various modulatory cascades that are affected in human cardiomyopathy. The cytosolic C terminus is a crucial domain that controls Calcium currents via interaction with a variety of regulatory proteins. We here report a novel protein termed Myoscape which is expressed in a highly striated muscle enriched fashion and which directly interacts with the cytosolic C-terminus. Immunolocalization of Myoscape reveals a distinct expression pattern colocalizing with Alpha-actinin at the sarcomeric z-disc as well as the L-type calcium channel at cardiac t-tubules. Adenoviral knockdown of Myoscape in cultivated adult rat ventricular cardiomyocytes (ARVCMs) leads to significant decrease in global calcium transients, with smaller calcium amplitudes lower diastolic calcium contents and a prolonged time to peak. Consistently, analysis of L-Type Calcium currents by patch clamp technique in ARVCM confirmed a significant reduction in L-Type channel open probability upon Myoscape ablation. Conversely, adenoviral overexpression of Myoscape significantly increases global Calcium transients in ARVCM and directly enhances L-Type Calcium channel currents. Moreover, overexpression of Myoscape is able to restore decreased L-Type Calcium channel currents in failing ARVCM. In contrast, adenoviral knockdown of Myoscape significantly reduced contractility of ARVCM. Finally, in vivo antisense-morpholino based knockdown of the Myoscape orthologue in zebrafish led to severe cardiomyopathy with impaired contractile function, pericardial edema and premature death. Of note, myocardial Myoscape protein abundance is significantly reduced in samples of patients who suffered from dilatative cardiomyopathy compared to control individuals.

Integrin-linked kinase (ILK)- Protein kinase B (PKB/Akt) signalling - a novel target for heart failure therapy

Presenting Author: Dr. Steffen Just / Sofia Hirth

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Most of the so far identified DCM disease genes encode for sarcomeric or cytoskeletal proteins, but the precise mechanisms that translate these mutations into the cardiomyopathic phenotype are still only poorly understood. In search for molecules that control cardiac contractility, we isolated the zebrafish mutant main squeeze (msq), which displays progressive heart failure due to a mutation (msqL308P) within the Integrin-Linked Kinase (ILK) gene. ILK is bound in a protein complex with Parvin and Pinch proteins, the so-called ILK-Pinch-Parvin (IPP) complex, and is known to have kinase activity that controls Protein kinase B (PKB/Akt) phosphorylation at Serine 473. The msq mutation interferes with kinase activity and its ability to bind b-Parvin. Accordingly, overexpression of active PKB rescues and knock-down of b-Parvin phenocopies the msq phenotype. We show that loss of Pinch leads to instability of ILK and heart failure. The cause of heart failure in PINCH morphants seems to be loss of PKB activity, since PKB phosphorylation at serine 473 is reduced and overexpression of active PKB reconstitutes cardiac function. These findings demonstrate that the ILK-PKB signaling pathways controls cardiomyocyte contractility, and when defective leads to DCM.
To investigate the signaling events that translate identified human mutations into DCM, we have generated transgenic fish lines inducibly (TetON/OFF) overexpressing human ILK mutations in a heart-specific manner. Furthermore, we isolated phosphatase inhibitors that enhance ILK signalling by stabilizing PKB phosphorylation. Using our high-troughput, fully-automated bioanalytics screening platform (robotics-assisted multicamera microscope), we are now able to easily screen the therapeutic relevance of small compound libraries on our defined zebrafish mutants in a 96- or 384-well format. Compounds that are able to rescue the msq phenotype will subsequently evaluated for their therapeutic potential in established heart failure models.

Transcriptome analysis identifies multifactorial mechanisms of cardiac remodeling and diastolic dysfunction

Presenting Author: Falko Matthes

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Intracellular Ca2+ leak due to ryanodine receptor (RyR2) dysfunction causes arrhythmias during catecholaminergic stress in mutation carriers. If RyR2 dysfunction contributes to maladaptive remodeling during sustained stress is unclear. We exposed knock-in mice with a patient mutation RyR2-R2474S/wt (RS) to catecholaminergic stress by continuous isoproterenol treatment for 3 to 28 days (osmotic pump ISO 20 mg/kg/d s.c.). Indeed, high resolution echocardiography identified a significant increase of cardiac wall thickness (wt 0.78±0.02 mm; RS 0.87±0.03 mm; each n=19, $p<0.05$) in the absence of any histological changes already 3 days after stimulation (3d) consistent with significant diastolic dysfunction.
Transcriptome analysis of isolated left ventricles occurred by microarray (Affymetrix Gene Chip Mouse 1.0 ST). Genome-wide data were analyzed by statistical methods (ANOVA modeling, non-linear local regression normalization, and component wise t-testing). Significant modifier mechanisms were identified early (3d) in RS hearts by canonical pathway analysis. Using threshold criteria (fold change >2.5; FDR<0.01) differential expression changes of Dilated Cardiomyopathy associated pathways were identified: the dystrophin complex (3.5-fold decrease), the glycosyl-transferase fukutin (4.4-fold decrease), the sarcomeric protein titin (3.9-fold decrease), and a myosin heavy chain (MHC) isoform shift (ß>a) consistent with contractile dysfunction. Furthermore, the RyR2 channel stabilizing protein triadin was significantly decreased in RS hearts at 3d, consistent with a significant increase in diastolic Ca2+ spark frequency, diastolic dysfunction, and cardiac remodeling.
Our data support a model where sustained catecholaminergic stress activates a powerful multifactorial mechanism of early cardiac remodeling. Using transcriptome analysis, we identify stress dependent molecular remodeling mechanisms of relevance for genetic (RS heart) and potentially common forms of heart disease.

High-fat feeding interacts with the Parkinson disease related protein DJ-1

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DJ-1 is associated with inherited and sporadic forms of Parkinson disease. Although the details of its biochemical function are still unclear, DJ-1 has been recognized as a multifunctional protein involved in the oxidative stress response, mitochondrial integrity, and transcriptional regulation amongst others. The intracellular DJ-1 protein pool comprises multiple isoforms varying in their isoelectric point (pI). Acidic forms, representing oxidized products, have been implicated in neurodegenerative diseases and cancer.
In a previous proteome analysis, DJ-1 was increased in the hypothalamus of mice after a short-term high-fat feeding. Here, we went on to quantify the individual DJ-1 isoforms. Therefore, we fed male C57BL/6N mice with a high-fat or control diet for 10 days and separated the pI isoforms in selected cerebral and peripheral tissues utilizing a high-throughput capillary-based isoelectric focusing system with subsequent immunodetection of DJ-1.
In hypothalamus, brain stem, cerebellum and fore brain, quantification revealed an increase in acidic DJ-1 isoforms in high-fat diet-fed mice relative to the control group while higher pI isoforms were significantly decreased. A high-fat diet-induced acidification of DJ-1 isoforms could also be detected in pancreas, muscle and liver but not in epididymal white adipose tissue. These results demonstrate that a high-fat diet causes intracellular changes that modify DJ-1. These modifications are similar to those observed under neurodegenerative conditions in human brain samples and cell lines under conditions of oxidative stress.
Moreover, we investigated the gene-diet interaction in Dj-1 deficient mice and show increased body fat accumulation in male Dj-1-/- mice after 14 weeks of high-fat feeding compared to their wildtype littermates while the lean mass is reduced.
Conclusively, we suggest that DJ-1 plays a role in the adaptation to a new metabolic situation.
This work is funded by NGFNplus (01GS0822).

Fat mass and obesity associated gene (FTO) regulates the expression of UCP-1 in human SGBS adipocytes

Presenting Author: Daniel Tews

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Introduction:
The association between gene variants in FTO (fat mass and obesity associated) has been shown in different genome-wide association studies. FTO encodes a 2-oxoglutarate dependent demethylase and is expressed ubiquitously. The phenotype of FTO deficient mouse models points to a participation of this gene in energy metabolism. However, its precise role in adipocyte metabolism has not been elucidated so far.

Objective:
To study the role of FTO in human adipocyte metabolism.

Methods:
By using lentiviral-mediated expression of shRNA, we generated FTO deficient SGBS pre- and adipocytes. Successful knockdown and expression of marker genes involved in adipogenic differentiation and glucose and lipid metabolism were monitored by qPCR. Relative mitochondrial content was determined by measurement of citrate synthase activity. Cellular oxygen consumption rates were analyzed by cell respirometry.

Results:
In human SGBS preadipocytes and adipocytes we reached a transduction efficiency of >90%. This led to an inhibition of FTO mRNA expression by 73% and to a total repression of FTO protein expression. FTO deficiency did not affect differentiation into mature adipocytes. Interestingly, expression of uncoupling protein 1 (UCP-1) was approximately 4-fold increased in mitochondria of FTO deficient adipocytes compared to preadipocytes. This led to an increased basal as well as uncoupled mitochondrial respiration in FTO deficient adipocytes.

Conclusions:
We conclude that FTO deficiency leads to the induction of a brown adipocyte phenotype, thereby enhancing energy expenditure. Further understanding of the signaling pathways connecting FTO with UCP-1 expression might lead to new options for obesity treatment.

The KDM4C histone demethylase is strongly expressed in neuroblastoma and maintains the undifferentiated state in vitro

Presenting Author: Annika Sprüssel

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Background: Epigenetic changes in DNA and histone methylation are hallmarks of most cancers. Several histone demethylases have been identified, most of which catalyze the removal of methyl groups from histone H3 lysine residues, thereby influencing gene expression. The KDM4C histone demethylase regulates H3K9me3 demethylation, and has been shown to work together with the epigenetically crucial KDM1A histone demethylase. A recent report from our own group implicates KDM1A -targeted therapy as a new option against neuroblastoma. Here we analyzed the role of KDM4C in neuroblastoma cell lines and its usefulness as a therapy target.
Methods: Cell viability, proliferation, death and differentiation were analyzed in the neuroblastoma cell lines, SHEP, SK-N-BE, SK-N-AS and IMR5, after siRNA-mediated KDM4C knockdown. In the same cell line panel, cell viability, proliferation and death were analyzed after inhibition of KDM4C, KDM1A or both using novel small molecule inhibitors.
Results: KDM4C knockdown significantly reduced cell viability and proliferation, and increased expression of neurotensin, a marker of neuronal differentiation. Similar results were obtained when KDM1A was inhibited using a novel small molecule inhibitor, whereas a small molecule inhibitor of KDM4C had no effect on cell viability in vitro. Combinatorial treatment with both inhibitors, however, produced synergistic effects on both cell viability and induction of differentiated cell morphology.
Conclusions: High levels of KDM4C expression in neuroblastoma cells are likely to contribute to the maintenance of an undifferentiated state in vitro. The combinatorial inhibition of KDM4C and KDM1A produces a synergistic effect on cell viability and a stronger induction of cell differentiation. These results indicate that integrating agents targeting cooperating pools of epigenetic regulators into molecular targeted therapy approaches could be beneficial for the treatment of neuroblastomas.

Lysine-specific demethylase 1 restricts hematopoietic progenitor proliferation and is essential for terminal differentiation

Presenting Author: Annika Sprüssel

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Lysine (K)-specific demethylase 1A (LSD1/KDM1A) has been identified as a potential therapeutic target in solid cancers and more recently in acute myeloid leukemia. However, the potential side effects of a LSD1-inhibitory therapy remain elusive. Here, we show, with a newly established conditional in vivo knockdown model, that LSD1 represents a central regulator of hematopoietic stem and progenitor cells. LSD1 knockdown (LSD1-kd) expanded progenitor numbers by enhancing their proliferative behavior. LSD1-kd led to an extensive expansion of granulomonocytic, erythroid and megakaryocytic progenitors. In contrast, terminal granulopoiesis, erythropoiesis and platelet production were severely inhibited. The only exception was monopoiesis, which was promoted by LSD1 deficiency. Importantly, we showed that peripheral blood granulocytopenia, monocytosis, anemia and thrombocytopenia were reversible after LSD1-kd termination. Extramedullary splenic hematopoiesis contributed to the phenotypic reversion, and progenitor populations remained expanded. LSD1-kd was associated with the upregulation of key hematopoietic genes, including Gfi1b, Hoxa9 and Meis1, which are known regulators of the HSC/progenitor compartment. We also demonstrated that LSD1-kd abrogated Gfi1b-negative autoregulation by crossing LSD1-kd with Gfi1b:GFP mice. Taken together, our findings distinguish LSD1 as a critical regulator of hematopoiesis and point to severe, but reversible, side effects of a LSD1-targeted therapy.

miR-542-3p exerts tumor suppressive functions in neuroblastoma by downregulating survivin

Presenting Author: Kristina Althoff

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Background: MicroRNAs (miRNAs) are deregulated in neuroblastoma (NB) and other cancers. We previously reported a signature of 42 miRNAs to be highly predictive for NB outcome. This signature included miR-542-3p, which was downregulated in tumors from patients with adverse outcome. Here we analyzed the influence of miR-542-3p expression on major aspects of NB tumor biology.

Methods: Pre-existing datasets (real-time miRNA expression, next-generation RNA sequencing and mRNA expression microarrays) from primary NBs were re-analyzed to assess expression of miR-542-3p, miR-542-5p and survivin. MiR-542-3p or -5p were re-expressed or survivin was downregulated in three NB cell lines, and resulting cell viability, proliferation and apoptosis were assessed. Reporter assays were performed. Combined overexpression of survivin and miR-542-3p was conducted in SHEP cells. NB xenografts in nude mice were intravenously treated with miR-542-3p nanoparticles.

Results: MiR-542-3p and miR-542-5p were expressed in equimolar ratios in primary NBs, and expression inversely correlated with poor prognosis. Re-expression of miR-542-3p in NB cell lines reduced cell viability and induced apoptosis and cell cycle arrest. Survivin was downregulated upon miR-542-3p re-expression in NB cell lines, and was inversely correlated with miR-542-3p expression in primary NBs. Reporter assays confirmed that miR-542-3p directly targeted survivin. Survivin knockdown phenocopied the effect of miR-542-3p re-expression, and combined overexpression of survivin and miR-542-3p partially rescued the miR-542-3p re-expression phenotype. Targeting NB xenografts in mice with miR-542-3p nanoparticles repressed expression of survivin and induced apoptosis.

Conclusions: MiR-542-3p exerts tumor suppressive functions in NB, at least in part, by targeting survivin. Initial evidence from miR-542-3p re-expression in vivo points to a promising potential of nanoparticle-targeted delivery of selected miRNAs as a novel therapy approach.

miR-137 functions as a tumor suppressor in neuroblastoma by downregulating KDM1A

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Background: The histone demethylase lysine-specific demethylase 1 (KDM1A, previously known as LSD1) is strongly expressed in neuroblastoma (NB), and its overexpression has been correlated with poor patient prognosis. Differentiation of NB cells resulted in down-regulation of KDM1A and siRNA-mediated KDM1A knockdown induced growth inhibition of NB cells. The microRNA miR-137 has been reported to be downregulated in several human cancers, and KDM1A was reported as a putative target of miR-137 in colon cancer. Based on these data, we hypothesized that miR-137 might have a tumor suppressive role in NB mediated via down-regulation of KDM1A.
Methods: MiR-137 and KDM1A expression was re-analyzed in pre-existing next-generation RNA sequencing and microarray expression data from 69 primary NBs. The effect of either KDM1A knockdown or miR-137 re-expression in the NB cell lines, SHEP, IMR-32 and SK-N-BE was assessed on cell viability, proliferation and apoptosis. Reporter assays were performed to assess miR-137 targeting of KDM1A.
Results: High levels of miR-137 expression in primary NBs were correlated with poor patient prognosis. Re-expression of miR-137 in NB cell lines increased apoptosis and decreased cell viability and proliferation. KDM1A was repressed by miR-137 in NB cells, and was validated as a direct target of miR-137 using reporter assays in SHEP and HEK293 cells. Furthermore, siRNA-mediated KDM1A knockdown phenocopied the miR-137 re-expression phenotype in NB cells.
Conclusions: We have shown that miR-137 directly targets KDM1A in NB cells, and activates cell properties consistent with tumor suppression. Therapeutic strategies focused on the re-expression of miR-137 in NBs could be useful to reduce NB aggressiveness.

A new MYCN-driven neuroblastoma mouse model using Cre-driven conditional expression of MYCN

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Background: In the first neuroblastoma (NB) mouse model, MYCN overexpression driven by a TH core promoter caused NB development. Although representing an excellent and broadly used tool, some limitations exist: 1. pronucleus injection with transgene integration into a less well-defined locus potentially yields less robust MYCN expression, 2. tumors predominantly originate from abdominal ganglion structures, 3. bioluminescent tumor imaging is impossible and 4. tumor incidence is 70% in the 129x1/SVJ but low in the C57Bl6 background. We aimed to overcome these limitations with a new mouse model using Cre-conditional MYCN expression in the neural crest.
Methods: The CAG-LSL-MYCN-IRES-Luciferase vector (LSL-MYCN) was introduced into the ROSA26 locus, and mice were crossbred with DBHiCre mice to target MYCN expression to the neural crest. Tumors were detected by bioluminescent imaging, characterized by histology, immunohistochemistry, PCR and western blotting.
Results: Abdominal tumors developed with almost 100% penetrance at 50-100 days of age in mice heterozygous for LSL-MYCN;DBHiCre in a mixed C57Bl6/129 strain background. Tumors were detectable by bioluminescent imaging, and arose predominantly from the adrenals, but occasionally also from superior cervical. They consisted of small round blue cells and expressed the NB tumor markers, TH, DBH and Phox2b. Several tumors were shown to originate from adrenal structures by histological analysis and by following tumor growth with high frequency ultrasound. The macroscopic tumor appearance, primary tumor sites, tumor histology and marker gene expression confirmed these tumors as NB, and western blotting confirmed strong MYCN expression.
Conclusions: Here we present a new NB mouse model with conditional expression of MYCN from a defined genomic locus in the neural crest. This model overcomes several limitations, and has the potential to improve investigations technically difficult for the current model.

CAMTA1 contributes to 1p36 tumor suppression

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A broad range of human malignancies is associated with non-random 1p36 deletions, suggesting the existence of tumor suppressors encoded in this region. In neuroblastoma, we identified a smallest region of consistent deletion that pinpoints the transcription factor gene CAMTA1. No evidence for somatic mutations was found but multivariate survival analysis identified low CAMTA1 mRNA expression as an independent predictor of poor outcome. In neuroblastoma cells that expressed little endogenous CAMTA1, its ectopic expression slowed cell proliferation, increasing the relative proportion of cells in G1/G0 phases of the cell cycle, inhibited anchorage-independent colony formation and supressed the growth of tumor xenografts. CAMTA1 also induced neurite-like processes and markers of neuronal differentiation in neuroblastoma cells. Transciptome analysis upon CAMTA1 induction revealed upregulation of genes involved in neuronal function and differentiation. Together, our findings define properties of CAMTA1 in growth suppression and neuronal differentiation that support its assignment as a 1p36 tumor suppressor gene in neuroblastoma. Similar to other 1p36 tumor suppressor candidates, including p73, CHD5, KIF1B, CASZ1 and miR-34a, CAMTA1 may not necessarily require biallelic inactivation in a classic "two-hit" manner but contribute to cancer development by partial dosage reduction via copy number loss or other mechanisms, including epigenetic inhibition. Functional studies indicate that these six tumor suppressor candidates cooperate to suppress tumorigenesis. Their codeletion may be one way for a developing cancer cell to acquire selective advantage by inhibiting an anti-oncogenic network at different positions in a single event. We propose a model where heterozygous 1p36 deletion impairs oncosuppressive pathways via simultaneous downregulation of several dosage-dependent tumor suppressor genes.

SplicingCompass: differential splicing detection using RNA-Seq data

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Alternative splicing is central for cellular processes and substantially increases transcriptome and proteome diversity. Aberrant splicing events often have pathological consequences and are associated with various diseases and cancer types. The emergence of next generation RNA sequencing (RNA-seq) provides an exciting new technology to analyze alternative splicing on a large scale. However, algorithms that enable the analysis of alternative splicing from short-read sequencing are not fully established yet and there are still no standard solutions available for a variety of data analysis tasks. We present a new method and software to predict genes that are differentially spliced between two different conditions using RNA-seq data. Our method employs geometric angles between the high dimensional vectors of exon read counts. With this, differential splicing can be detected even if the splicing events comprise of higher complexity and involve previously unknown splicing patterns. We applied our approach to two case studies including neuroblastoma tumour data with favourable (stage 4s) and unfavourable (stage 4) clinical courses. We show the validity of our predictions as well as the applicability of our method in the context of patient clustering. We verified our predictions by several methods including complementary in silico analyses of the data itself. We found a significant number of exons with specfic regulatory splicing factor motifs for predicted genes and a substantial number of publications linking those genes to alternative splicing. Furthermore, we could successfully exploit splicing information to cluster tissues and patients. Finally, we found additional evidence of splicing diversity for many predicted genes in normalized read coverage plots and in reads that span exon-exon junctions.

MYCN or ALKF1174L are sufficient to drive neuroblastoma development from neural crest progenitor cells

Presenting Author: Sven Lindner

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Background: Few recurrent genetic changes contributing to neuroblastoma (NB) formation, such as amplification of MYCN and activating mutations of the ALK gene, have been identified. NB is presumed to arise from the neural crest, but models demonstrating its development from neural crest progenitor cells are still missing. Here we present direct evidence that neural crest progenitor cells give rise to NB using a multipotent murine neural crest progenitor cell line, JoMa1.
Methods: The JoMa1 cell line was maintained in an undifferentiated state by a tamoxifen-activated c-Myc transgene (c-Myc-ERT). Either MYCN, the oncogenic F1174L ALK variant identified in primary NBs, TrkA or GFP were ectopically expressed in JoMa1 cells. Subsequently, growth independence of c-Myc-ERT transgene was monitored in vitro, and tumorigenicity of transfected cells was evaluated in nude mice.
Results: JoMa1 cells expressing MYCN or ALKF1174L were able to grow independently of c-Myc-ERT activity in vitro and formed tumors in nude mice, as opposed to parental JoMa1 cells and JoMa1 cells expressing TrkA or GFP. Tumors resembled human NBs in morphology, and expressed the NB markers, TH, DBH, Phox2b, CD56 and synaptophysin. In addition, neurosecretory vesicels and synaptic structures were visible in electron micrographs of the tumors. Tumorigenicity was enhanced upon serial transplantation of tumor-derived cells as subcutaneous grafts in nude mice. MYCN-driven tumor cells remained susceptible to the MYC-inhibitor, NBT-272, indicating that cell growth depended on functional MYCN.
Conclusion: Our findings support neural crest progenitor cells as the precursor cells of NB. The JoMa1 model system is a well validated and rapid tool to investigate genes suspected of contributing to neuroblastomagenesis in a mouse model or implement screening strategies to identify such genes. The former strategy has already been successfully applied for FOXR1, LIN28b and miR-17-92.

MINA53 expression in neuroblastoma correlates with unfavorable patient outcome

Presenting Author: Andrea Odersky

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Background: The MYC-induced nuclear antigen (MINA) is overexpressed in many cancer types, including HCC and lung cancer. MINA is induced by cMYC and has been shown to exert a strong effect on proliferation of these tumor cells. Like many demethylases and methyl-histone interacting proteins, the MINA protein harbors a JmjC domain, implicating MINA as a possible regulator of chromatin structural modulation and transcription. We here aimed to analyze the role of MINA expression in neuroblastoma (NB).
Methods: MINA expression was analyzed in 88 primary NB using Affymetrix microarrays, and confirmed using RT-qPCR in an independent NB cohort of 351 NB. MINA expression was analyzed by RT-qPCR and western blotting in a NB cell line panel as well as the tamoxifen-inducible SHEP-NMYC-ER cell model. Transient (siRNA) and stable (shRNA) MINA knockdown was carried out in the NB cell lines Kelly and IMR32, and cell viability, proliferation and apoptosis was subsequently assessed.
Results: High MINA expression in primary NBs was significantly inversely correlated with event-free and overall survival in patients ($p < 0.001$) and strongly positively correlated with MYCN amplification in the tumors ($p < 0.001$). These results were confirmed in an independent cohort of 351 NB using RT-qPCR. High expression levels of MINA were confirmed in a panel of 4 amplified and 4 nonamplified NB cell lines using RT-qPCR and western blotting. MINA expression was also strongly induced by MYCN activation in SHEP-NMYC-ER cells. Targeting MINA expression by siRNA and shRNA significantly decreased cell viability and proliferation of NB cells and induced massive apoptosis.
Conclusion: MINA is a new target gene regulated by MYCN in NB cells, and MINA expression in primary NBs has prognostic value for NB patients. It has potential as a drug target circumventing the need to target MYCN directly in MYCN-dependent tumors, and warrants further functional investigation.

Profiling of proteome changes induced by neuroblastoma master regulators

Presenting Author: Alexander Schramm

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Neuroblastoma (NB) is a common pediatric solid tumor, which displays striking clinical and biological heterogeneity. Genomic sequencing has revealed that no single genetic change can be attributed to NB. Up to now, amplification or point mutation of oncogenes including ALK and LIN28B was found to be causally involved in NB tumorigenesis. Still, for the majority of NBs, the key events and the tumor driving forces remain to be explored. Common to all NBs is that they arise from neural crest progenitors. In normal development, the activity of the transcription factors MYCN and SOX10, among others, control the cellular fate of this lineage. Deregulation of the MYCN- and SOX10-regulated pathways are thought to play a pivotal role during NB formation. Here, we attempted to gain insights into the interaction partners of the transcription factors SOX10 and MYCN in human neuroblastoma SY5Y cells using proteomic profiling. For this purpose we deployed affinity-based purification of protein complexes and subsequent mass spectrometry. To confirm specificity of interaction partners, isotope labeling of proteins (SILAC) was established in SY5Y cells stably expressing SOX10. In total, we identified 22 interaction partners of SOX10 and we are currently validating the most promising candidates.

miR-34a is dispensable for normal development but its loss accelerates medulloblastoma formation

Presenting Author: Theresa Thor

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MicroRNAs inhibit translation or initiate degradation of target mRNA by sequence-specific interaction and are involved in regulating important functions in development and differentiation. Previous studies have evaluated the role of miRNAs in the initiation and progression of cancer. MiR-34a was found to be downregulated in several tumor entities, including medulloblastoma. Our analysis of miR-34a expression in primary medulloblastoma and medulloblastoma cell lines revealed significant downregulation compared to normal human cerebellum. Reexpression of mir-34a in human medulloblastoma cells in vitro reduced cell viability and proliferation and induced apoptosis. Among the targets we found downregulated by miR-34a in human medulloblastoma cells were MYCN and SIRT1. The well-established medulloblastoma mouse model ND2:SmoA1 is based on activation of the SHH pathway by transgenical expression of a constitutively active form of Smoothened in mouse GNPs. Analysis of miR-34a in ND2:SmoA1-derived medulloblastoma revealed significant suppression of miR-34a compared to samples of murine cerebellum. We analyzed miR-34a function in vivo by targeted transgenesis in mice. As expected, tissues from mice with constitutive homozygous deletion of the miR-34a gene did not express miR-34a transcripts as determined by qRT-PCR. Mice were viable, fertile and a comprehensive standardized phenotype analysis performed by the German Mouse Clinic revealed only minor effects in respect to the phenotype. Crossbreeding ND2:SmoA1 mice with miR-34a knockout mice significantly accelerated medulloblastoma growth in mice deficient for miR-34a. Interestingly, MYCN and SIRT1 were much more strongly expressed in medulloblastoma derived from these mice. Taken together, we demonstrate that miR-34a is dispensable for normal development, but that its loss accelerates medulloblastoma formation in mice. Strategies aiming to re-express miR-34a in tumors could be effective therapeutic strategies in the future.

ALKF1174L is a driving oncogene of neuroblastoma in transgenic mice

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Activating anaplastic lymphoma kinase (ALK) mutations occur in most familiar and in 10% of sporadic neuroblastoma, but the role of mutated ALK in tumorigenesis remains elusive. We here demonstrate that targeted expression of the most frequent and aggressive variant, ALKF1174L, is tumorigenic in mice.

Transgenic mice were generated by pronucleus injection of the CAGCS-LSL-ALKF174L-IRES-Luciferase vector. Founders were crossbred with DBHiCre mice to target expression of ALK to the neural crest and with TH-NMYC mice to explore synergism between ALKF1174L and MYCN. Tumors were characterized using histology, immunohistochemistry, electron microscopy, PCR, western blotting, aCGH and mRNA microarrays. Transgenic tumor-bearing mice and nude mice carrying human NB xenografts were treated with either crizotinib or NVP-TAE-684.

Of the mice transgenic for LSL-ALKF1174L and DBHiCre, 5 of 12 developed tumors between 130 and 351 days of age. Tumors resembled human NB in morphology, metastasis pattern, gene expression and subcellular structures. aCGH revealed that this ALK-driven NB mouse model recapitulated the genetic spectrum of human NB. Chromosomal aberrations were syntenic to those in human NB, including 17q gain and MYCN amplification. Targeted ALKF1174L and MYCN co-expression revealed a strong synergism in inducing NB with minimal secondary hits. Treatment of ALKF1174L transgenic mice with the NVP-TAE-684 ALK inhibitor induced complete tumor regression. NVP-TAE-684 treatment of SY5Y-derived xenografts afforded similar results in contrast to crizotinib, which failed to successfully treat NB xenografts harboring ALKF1174L.

We conclude that an activating mutation within the ALK kinase domain is sufficient to drive NB, and selected ALK inhibitors show promise to treat NB harboring ALK mutations. Since this model recapitulates all major genetic subtypes, it offers unique opportunities for NB research and will be of particular importance to explore ALK-targeted therapy of NB.

Lysin-specific histone demethylase 1 (LSD1) is a promising new epigenetic therapy target crucially involved in the migration of medulloblastoma cells

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Epigenetic modifications including DNA methylation and histone acetylation are thought to play important roles in the onset and progression of cancer including brain tumors. More recently, also histone methylation has been identified as a major determinant of oncogenesis as well as embryonal development and differentiation. The prototype of the new class of histone demethylating enzymes is LSD1, which controls broad gene expression programs and is involved in malignant progression of several cancers. As enzymes controlling epigenetic alterations are of considerable interest as targets for cancer therapy, we here examined the role of LSD1 in medulloblastoma. Re-analysis of microarray data and immunohistochemical analysis of 87 medulloblastomas in a tissue microarray revealed upregulation of LSD1 mRNA and protein in medulloblastomas compared to normal cerebellar tissue. High LSD1 transcript levels in primary medulloblastomas positively correlated with reduced overall patient survival. LSD1 was also strongly expressed in human medulloblastoma cell lines and medulloblastomas derived from PTCH+/-/SmoA1 mice. Interestingly, downregulation of LSD1 in human medulloblastoma cells using siRNA significantly reduced cell migration. Furthermore, LSD1 expression increased in the distinct phase of granule precursor cell migration during cerebellar development in mice. BMP2 was identified as a major LSD1 target gene, as it was most prominently up-regulated upon LSD1 knockdown. Consequently, the Smad signaling pathway was reactivated in LSD1-knockdown cells. We conclude that LSD1 plays a crucial role for the migration of both MB and potential MB precursor cells, suggesting a deregulation of a physiological function of LSD1 during MB development. This work lays the foundation for further preclinical and clinical evaluations of LSD1 as a promising new epigenetic therapy target for MB.

Pharmacological activation of the p53 pathway by nutlin-3 exerts anti-tumoral effects in medulloblastomas.

Presenting Author: Theresa Thor

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Medulloblastomas account for 20% of pediatric brain tumors. With an overall survival of 40%-70%, their treatment is still a challenge. The majority of medulloblastomas lack p53 mutations, but even in cancers retaining wild-type p53, the tumor surveillance function of p53 is inhibited by the oncoprotein MDM2. Deregulation of the MDM2/p53 balance leads to malignant transformation. Here, we analyzed MDM2 mRNA and protein expression in primary medulloblastomas and normal cerebellum and assessed the mutational status of p53 and MDM2 expression in 6 medulloblastoma cell lines. MDM2 expression was elevated in medulloblastomas, compared with cerebellum. Four of 6 medulloblastoma cell lines expressed wild-type p53 and high levels of MDM2. The tumor-promoting p53-MDM2 interaction can be inhibited by the small molecule, nutlin-3, restoring p53 function. Targeting the p53-MDM2 axis using nutlin-3 significantly reduced cell viability and induced either cell cycle arrest or apoptosis and expression of the p53 target gene p21 in these 4 cell lines. In contrast, DAOY and UW-228 cells harboring TP53 mutations were almost unaffected by nutlin-3 treatment. MDM2 knockdown in medulloblastoma cells by siRNA mimicked nutlin-3 treatment, whereas expression of dominant negative p53 abrogated nutlin-3 effects. Oral nutlin-3 treatment of mice with established medulloblastoma xenografts inhibited tumor growth and significantly increased survival. Thus, nutlin-3 reduced medulloblastoma cell viability in vitro and in vivo by re-activating p53 function. We suggest that inhibition of the MDM2-p53 interaction with nutlin-3 is a promising therapeutic option for medulloblastomas with functional p53 that should be further evaluated in clinical trials.

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

Presenting Author: Andrea S. Bauer

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Pancreatic cancer is the fifth most frequent cause of cancer-related death in industrialized countries. Early diagnosis would have an enormous impact on the successful treatment of pancreatic cancer, for which currently the incidence is nearly identical to mortality. In this study, variations in the abundance of mRNA and miRNA molecules in total RNA preparations from pancreas tissues and peripheral blood cells were analysed on comprehensive microarrays and validated by real-time PCR.
From blood, a clear distinction between healthy people and patients with either chronic pancreatitis or cancer could be achieved. However, no discrimination was possible between the diseases. This was accomplished by studying the mRNA and microRNA signatures obtained from tissue samples. Therefore, once a medical indication of disease has been established, more discriminative diagnosis can be performed by an analysis of RNA signatures of tissue samples.
Our overall aim is the combination of data obtained by RNA- and Proteome-based profiling and Epigenetic studies based on the identical set of hundreds of tissue samples of pancreatic cancer patients. 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.

Novel role of Fas-activated serine/threonine kinase (FASTK) in proliferation and survival of pancreatic cancer cells.

Presenting Author: Brajesh P. Kaistha

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We performed highly parallelized functional assays to identify novel target genes having key pathophysiological roles in pancreatic cancer. Reverse transfection microarrays were used to examine the functional effects of overexpression and knockdown of 88 pre-selected candidate genes in cancer and control cells. 19 candidates producing significant and reproducible effects were selected for further in-depth characterization.
This project aims to functionally characterize one of the selected genes, Fas-activated serine/threonine kinase (FASTK). Although FASTK was first described in mid nineties and has been shown to be implicated in regulation of apoptosis in certain cancer types, the exact physiological functions of this gene remain largely obscure, especially with respect to PDAC.
qRT-PCR data confirmed the overexpression of FASTK in pancreatic cancer tissues and further demonstrated that strong FASTK expression is also retained in the majority of pancreatic tumor cell lines in vitro. Functional effects of FASTK were investigated after transient knockdown in a variety of transformed and non-transformed cell lines. Proliferation (BrdU incorporation assay), viability (MTT assay) and clonogenic potential (Anchorage independent growth assay) of the cancer cells were significantly impaired by down-regulation of FASTK. Western blot analyses showed activation of apoptosis pathways (Caspase-3 and PARP cleavage). Further experiments involving generation of stable inducible (pTRIPZ shRNA) clones for further in vivo analyses as well as experiments for deciphering the pathways involved are currently in progress.
In conclusion, our experimental data show that knockdown of gene FASTK severely influences proliferation and viability of pancreatic cancer cells and results in significantly reduced resistance to apoptosis.

Characterization of crude and affinity-purified exosomes from pancreatic cancer cells

Presenting Author: Susanne Klein-Scory

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Exosomes are small microvesicles (30-100 nm) released from most normal, diseased, and neoplastic cells. Studies have demonstrated that exosomes play a role in cell communication and carry a modulatory role to cell growth and immune responses. Here we present the proteomic description of affinity purified exosomes from pancreatic tumor cells compared to the secretome, defined as the whole of proteins released by pancreatic cancer cells. The proteomic data provided comprehensive catalogues of hundreds of proteins. The comparison revealed a special proteomic composition of pancreatic cancer cell derived exosomes. Proteins derived from proteasomes and ribosomes as well as metabolic enzymes were lost upon affinity purification, whereas membrane proteins, glycoproteins, small GTP binding proteins and a heterogeneous group of further proteins were enriched. Proteins playing a role in tumorigenesis and modulators of ECM or cell-cell interactions were specifically enriched in affinity purified exosome preparations. Nucleic acid binding proteins represent another significant fraction of the exosomal preparations. These data are consistent with the finding that exosomes are also carriers of RNAs, especially of small RNAs. Profiling of the RNA content of exosomal preparations is ongoing.

Subscan - a cluster algorithm for identifying statistically dense subspaces

Presenting Author: Johann M. Kraus

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Cluster analysis is an important technique of initial explorative data mining. It refers to a collection of statistical methods for learning the structure of data by solely exploring pairwise distances or similarities in feature space. Recent approaches in clustering aim at detecting groups of data points that exist in arbitrary, possibly overlapping subspaces. Generally, subspace clusters are neither exclusive nor exhaustive, i.e. subspace clusters can overlap as well as data points are not forced to participate in clusters. In this context subspace clustering supports the search for meaningful clusters by including dimensionality reduction in the clustering process. Subspace clustering can overcome drawbacks from searching groups in high-dimensional data sets, as often observed in clustering biological or medical data. In the context of microarray data this refers to the hypothesis that only a small number of genes is responsible for different tumor subgroups. We generalize the notion of scan statistics to multi-dimensional space and introduce a new formulation of subspace clusters as aggregated structures from dense intervals reported by single axis scans. Our approach objectifies the search for subspace clusters as the reported clusters are of statistical relevance and are not artifacts observed by chance. Like in hierarchical cluster analysis there are two possible strategies to detect relevant subspace clusters. In a top-down approach, the dimension of clusters identified in the full space is reduced until a minimal subspace supporting the cluster assumption is reached. Using a bottom-up strategy allows the agglomeration of clusters from regions of high density across intervals from different dimensions. We present a bottom-up algorithm to grow high-dimensional subspace clusters from one-dimensional statistically dense seed regions. Our experiments demonstrate the applicability of the approach to both low-dimensional as well as high-dimensional data.

High-throughput analyses of cancer candidate genes reveals a central role for Cofilin-1 (CFL1) in regulation of cell proliferation in pancreatic cancer.

Presenting Author: Sandra Melchisedech

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Within the context of the NGFN PaCaNet consortium, we performed highly parallelized functional analyses of 80 pre-selected pancreatic cancer candidate genes. Overexpression and knockdown experiments in different cell lines were performed via 'reverse transfection' and influences on cell functions examined by fluorescence microscopy and immunocytochemical staining.
Here, we focus on cofilin-1 (CFL1), an actin-modulating protein which binds and depolymerizes filamentous F-actin. CFL1 has a well-studied role in cell migration; however, the results of our parallelized assays indicated that CFL1 also has a previously undocumented role in growth regulation of pancreatic cancer cells. This project thus aimed at examining the functions of CFL1 in pancreatic adenocarcinoma in detail.
Histological staining confirmed a significant overexpression in pancreatic cancer tissues compared to normal pancreatic ducts or chronic pancreatitis. Functional assays were performed after transient overexpression or knockdown of CFL1 in carcinoma cell lines (e.g. PANC-1, S2-007) and non-transformed cells (HEK293). BrdU- and MTT-assays demonstrated a significant reduction of cell proliferation after transient downregulation of CFL1 in 4 different cancer cell lines. Apoptosis was not induced, as shown by Western Blot for Caspase-3 and PARP cleavage. Additionally, knockdown experiments showed a decreased ability for anchorage-independent growth in soft agar assays and altered organization of the actin cytoskeleton, as demonstrated by phalloidin staining. Furthermore, a reduced migratory potential after knockdown of CFL1 was apparent in time lapse analyses and wound healing assays.
Our results thus demonstrate for the first time a direct growth regulatory function of CFL1 in cancer. Further experiments will include the generation of stable knockdown clones for in vivo experiments and are currently in process.

Functional analysis of TMPRSS2:ERG fusion gene variants in prostate cancer cell lines

Presenting Author: Mark Laible

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The TMPRSS2-ERG fusion gene is found in ~50% of all prostate cancers. This fusion event results in high expression of the transcription factor ERG as it is set under the control of the androgen responsive TMPRSS2 promoter. Fusion positive tumors show a clearly altered gene expression pattern and therefore represent a distinct subtype of prostatic tumors. However, it is not yet well understood which role the fusion event plays in tumor formation and whether it confers a growth advantage. Recent studies have indicated the involvement of ERG in the process of EMT. Furthermore, several variants of the TMPRSS2:ERG (T/E) fusion gene have been described. Within the ERG positive subgroup tumors with a T/E VI variant showed a more aggressive phenotype than tumors carrying other T/E variants. To study differences in the molecular effects of T/E variants, we chose to compare three T/E variants by inducible overexpression in the prostate cell lines LNCaP and RWPE1.
Successful overexpression was confirmed by Western blot and the functionality of the ORFs was evaluated by measuring mRNA levels of known ERG downstream targets. A set of genes deregulated in T/E tumors were selected from a previous expression profiling. mRNA levels of these genes was measured after induction of ERG in the cell lines. Most of the candidate genes were regulated in the same direction as expected based on the microarray data, indicating a good concordance of the in vitro model with the regulatory function of ERG in prostate tumors. Further cell culture experiments showed a decrease in viability upon ERG overexpression in LNCaP and RWPE1. Accompanying this drop in viability, LNCaP cells exhibited strong morphological changes, characterized by loss of cell-cell adhesion. In summary, we have created a model for studying induction of EMT-like characteristics by the ERG transcription factor. Further experiments will focus on the differences found between the T/E variants.

miRNA deregulation by aberrant promoter methylation in prostate cancer

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miRNAs are important regulators of gene expression and involved in tumorigenesis and disease progression. Recent publications have demonstrated that epigenetic changes, i.e., promoter methylation, largely contribute to miRNA deregulation in cancer. The aim of this study is to investigate the impact of promoter methylation on miRNA expression in prostate cancer (PCa), the most common malignant tumor in males and the third leading cause of cancer-related deaths in Western countries.
Based on two genome-wide miRNA expression datasets of altogether 58 PCa and 49 normal prostate tissues generated by Next generation sequencing and quantitative PCR, as well as published studies, we identified 24 miRNAs that are consistently downregulated and 17 miRNAs that are consistently upregulated in PCa. Putative promoter regions for these miRNAs were extracted from several published studies. Analyses of matched genome-wide DNA methylation data of the two datasets mentioned above revealed 29 miRNAs (71 %) that carry differentially methylated regions (DMRs) within 36 putative promoter regions in both datasets (1 - 8 DMRs per miRNA). Methylation of these DMRs was decreased and miRNA expression increased upon 5-aza-2'-deoxycytidine treatment of PCa cells indicating regulation of these transcripts by aberrant methylation of the putative promoters. Ongoing technical validation using 35 PCa and 35 normal prostate tissues by MassARRAY confirmed aberrant methylation in 80 % of the DMRs analyzed so far, accompanied by a negative correlation with the expression of the respective miRNA. Furthermore, analysis of an independent genome-wide methylation dataset (TCGA) confirmed aberrant methylation for 13 of 14 DMRs covered by these data. Taken together, our integrative study using several independent large datasets demonstrates that aberrant methylation of putative miRNA promoters in PCa is frequent and contributes to miRNA deregulation.

A novel splice variant of Calcium and Integrin Binding Protein 1 mediates protein kinase D2 stimulated tumour growth and angiogenesis

Presenting Author: Milena Armacki

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Protein kinase D2 (PKD2) is a member of the PKD family of serine/threonine kinases, a subfamily of the CAMK super-family. PKDs play a critical role in cell motility, migration, and invasion of cancer cells. Expression of PKD isoforms is deregulated in various tumours and PKDs, in particular PKD2, have been implicated in the regulation of tumour angiogenesis.
For a better understanding of the precise signalling context of PKD2, in particular with respect to angiogenesis, we used in vitro expression cloning to identify potential novel PKD substrates that could have an impact on angiogenesis. One potentially novel substrate identified in the screen was a splice variant of human Calcium and Integrin Binding protein 1 (CIB1) that we termed CIB1a.
CIB1 is a widely expressed protein that has been implicated in angiogenesis, cell migration, and proliferation, all important hallmarks of cancer, and CIB1a was found to be highly expressed in various cancer cell lines. We identified Ser118 as the major PKD2 phosphorylation site in CIB1a and showed that PKD2 interacts with CIB1a via its alanine and proline rich domain. Furthermore, we confirmed that CIB1a is indeed a substrate of PKD2 also in intact cells using a phosphorylation specific antibody against CIB1a-Ser118. Functional analysis of PKD2-mediated CIB1a phosphorylation revealed that upon phosphorylation, CIB1a mediates tumour cell invasion, tumour growth and angiogenesis. Thus, CIB1a is a novel mediator of PKD2-driven carcinogenesis and a potentially interesting therapeutic target.

Circulating microRNAs as Surrogate Markers for Circulating Tumour Cells and Prognostic Markers in Metastatic Breast Cancer

Presenting Author: Dharanija Madhavan

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Purpose: The utility of circulating tumour cells (CTCs) as a prognostic marker in metastatic breast cancer (MBC) has been well-established. However, their efficacy and accuracy are still under scrutiny mainly due to methods of their enrichment and identification. We hypothesized that circulating microRNAs (miRNAs) can predict the CTC status of MBC patients, and tested for the same. Furthermore, we aimed at establishing a panel of circulating miRNAs capable of differentiating MBC cases from healthy controls.

Experimental design: Circulating miRNAs from plasma of CTC-positive and CTC-negative MBC patients, and healthy controls were profiled by TaqMan Human MicroRNA arrays. Candidates from the initial screen were validated in an extended cohort of 269 individuals (61 CTC-positive, 72 CTC-negative, 60 CTC-low MBC cases and 76 controls).

Results: CTC-positive had significantly higher levels of miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-210, miR-375 and miR-801 than CTC-negative MBC and controls ($P < 0.00001$), while miR-768-3p was present in lower amounts in MBC cases ($P < 0.05$). miR-200b was singled out as the best marker for distinguishing CTC-positive from CTC-negative patients (AUC 0.88). We identified combinations of miRNAs for differentiating MBC cases from controls (AUC 0.95 for CTC-positive; AUC 0.78 for CTC-negative). Combinations of miRNAs and miR-200b alone were found to be promising prognostic marker for progression-free and overall survival.

Conclusion: This is the first study to document the capacity of circulating miRNAs to indicate CTC status and their potential as prognostic markers in MBC patients.

Identification of microRNAs targeting specific PAR-CLIP clusters

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PAR-CLIP is a high-throughput method to identify binding sites of RNA binding proteins. This is done by immunoprecipitation (IP) of the protein of interest and deep sequencing the RNA crosslinked to proteins prior to IP. PAR-CLIP also has extensively been used to determine target sites of microRNAs by isolating their binding protein AGO. In an AGO-PAR-CLIP experiment, the identity of the microRNA responsible for a target site is a priori not clear and must be revealed by matching the microRNA seed sequences to the target site sequence, which is not a trivial task.

PAR-CLIP data has several specific characteristics, most notably, frequent T to C conversions that are indicative for crosslinking sites. We utilize these and other features to accurately determine the seed site.

Our method, PARma, consists of two main components: A generative model incorporates PAR-CLIP specific features to compute likely seed sites and the novel pattern discovery tool kmerExplain estimates seed activity probabilites based on the likelihood inferred by the model. KmerExplain can estimate seed activities without a predefined set of microRNAs, which allows to detect active regulators in an unbiased way, but can also incorporate prior probabilies for microRNA seeds. Both components, model estimation and kmerExplain are iteratively applied until convergence.

The final PAR-CLIP model is in agreement with known binding mechanisms of microRNAs and with structural knowledge of AGO and many active k-mers correspond to seeds of expressed microRNAs. The final seed assignment has two properties: Each active seed sequence explains several clusters with high likelihood and the seed positions match the model of PAR-CLIP data learned from all target sites. Based on evaluations using differential PAR-CLIP data from both a publicly available dataset as well as from a new dataset, we show that PARma is more accurate than existing approaches in terms of correct seed assignments.

From SARS-Coronavirus (SARS-CoV) Genome to Cellular Targets and Pan-coronaviral Replication Inhibition

Presenting Author: Albrecht von Brunn

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Coronaviruses (CoV) are important human and animal pathogens that induce fatal respiratory, gastrointestinal and neurological disease. The outbreak of the severe acute respiratory syndrome (SARS) in 2002/2003 has demonstrated human vulnerability to CoV epidemics. Neither vaccines nor therapeutics are available against human and zoonotic CoV. Knowledge of host cell proteins that take part in pivotal virus-host interactions could define novel broad-spectrum antiviral targets. Using a systems-biology approach, we identified in this study by genome-wide yeast-two hybrid interaction screening cyclophilins as interaction partners of the CoV non-structural protein 1 (Nsp1, (Pfefferle et al., 2011, PLoS Pathog 7: e1002331). Interactions of Nsp1 with cylophilins modulated the Calcineurin/NFAT pathway and led to altered cytokine secretion, a key feature of viral immunopathogenesis. Conversely, inhibition of cyclophilins, integral components of the NFAT pathway when complexed with Cyclosporine A (CsA), inhibited replication of CoV of all genera, including SARS-CoV, human CoV-229E and -NL-63, feline CoV, porcine transmissible gastroenteritis virus [TGEV] as well as avian infectious bronchitis virus. Non-immunosuppressive derivates of CsA could serve as broad-range coronaviral inhibitors against emerging new CoV and ubiquitous pathogens of humans and livestock as well.

ChroMoS: a web-tool for SNP classification and prediction of their regulatory potential based on genome segmentation data

Presenting Author: Maxim Barenboim

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Genome-wide association studies (GWAS) reveal increasing number of disease-associated SNPs. However, since majority of these SNPs are located in intergenic and intronic regions the assessment of their functionality was hindered by the lack of information about regulatory regions. This problem is aggravated by the large amount of deep-sequencing data emerging from clinical studies. It requires an automated SNP prioritization for initial analysis to be followed by more focused functional analysis. In parallel efforts, epigenetics studies have resulted in many publicly available ChIP-seq profiles that allow segmentation of the genome into chromatin states with various functions.

Here we describe a web server, ChroMoS (Chromatin Modified SNPs), which combines genetic and epigenetic data with the goal to facilitate SNP classification and prioritization. To this end the user can provide SNP data in VCF format or select annotated SNPs from our MySQL database, which includes dbSNPs and data from many published GWAS studies. Next the user provides annotations for chromatin state regions, which are either user-defined (bed-files) or obtained from pre-calculated segmentation of epigenomic data for specific cell types that are stored in our database. The genome segmentation based on chromatin marks allows predictions of functional elements, such as enhancers and promoters. Based on this data ChroMoS suggests the functional impact of a SNP. In the process, SNPs are classified as non-coding and coding SNPs, and assigned to the various chromatin states. It also can be downloaded in flat format for downstream analysis. SNPs positioned in enhancer or transcription states are automatically subjected to differential analysis of transcription factor binding and for SNPs with potential impact on post-transcriptional mechanisms we use MicroSNiPer to assess the differential binding capacity of annotated miRNA.

Integrative Disease Gene Prioritization using NetworkPrioritizer

Presenting Author: Tim Kacprowski

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The identification and characterization of candidate disease genes are important for understanding the molecular foundations of human diseases. To select candidates for follow-up experiments, computational prioritization methods exploit the available biomedical knowledge. The recently introduced MedSim method showed that high performance can already be achieved using functional annotations alone [1]. However, the limitations of single data sources, e.g. incomplete or low-quality annotations, have led to recent prioritization approaches that consider multiple data sources [2]. Either a ranking of candidates is derived from integrated data or multiple data source-specific rankings are aggregated. Nevertheless, most freely available prioritization methods do not allow the user to modify the data integration process. Furthermore, the data used for the prioritization are often pre-defined by the method and not extensible by the user.
Our new Cytoscape plugin NetworkPrioritizer analyzes heterogeneous molecular networks and enables the user to choose which data sources to integrate and how to combine them into networks. The candidates are ranked according to their relevance for the disease based on network connectivity, which is estimated by different centrality measures. Each measure generates another ranking of candidates. To merge the different rankings, NetworkPrioritizer provides various rank aggregation algorithms. A leave-one-out cross-validation showed excellent performance of NetworkPrioritizer. Its application to recently published susceptibility loci for Crohn's disease resulted in a number of putative disease genes for follow-up experiments.

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microRNA-31 sensitizes human breast cells to apoptosis by direct targeting of protein kinase C epsilon (PKCe)

Presenting Author: Cindy Körner

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MicroRNAs (miRNAs) post-transcriptionally regulate gene expression and thereby contribute to the modulation of numerous complex and disease-relevant cellular phenotypes including cell proliferation, cell motility, apoptosis and stress response. In breast cancer cell systems miR-31 has been shown to inhibit cell migration, invasion and metastasis. Here, we link enhanced expression of miR-31 to the inhibition of the oncogenic NF-κB pathway thus supporting the tumor-suppressive function of this miRNA. We identified protein kinase C epsilon (PKCe encoded by the PRKCE gene) as novel direct target of miR-31 and show that downregulation of PKCe results in impaired NF-κB signalling, enhanced apoptosis and increased sensitivity of MCF10A breast epithelial and MDA-MB-231 triple-negative breast cancer cells towards ionizing radiation as well as treatment with chemotherapeutics. Mechanistically, we attribute this sensitization to anti-cancer treatments to the PRKCE-mediated downregulation of the anti-apoptotic factor BCL2. In clinical breast cancer samples, we observed an association of high BCL2 expression with poor prognosis. Further we found an inverse correlation of miR-31 and BCL2 expression, highlighting the functional relevance of the indirect downregulation of BCL2 via direct targeting of PRKCE by miR-31.

DiGtoP: From Disease Genes to Protein Pathways

Presenting Author: Svenja Hensler

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Remarkable progress in human genetics has associated specific genetic variations with numerous diseases. However, making a genetic association to a disease is often insufficient to understand the molecular underpinnings of disease etiology. Understanding disease mechanisms remains the major challenge.

Proteomic mapping offers a way forward by identifying physical protein relationships and indicating pathways, which can be validated by functional assays and offers novel targets for therapeutic interventions.

The goal of DiGtoP is to determine the physiological proteome environment of disease candidate genes associated with cancer, leukaemia, diabetes, obesity, schizophrenia, depression, Parkinson's and Alzheimer's disease in human and murine ES cells and human HeLa cells in vitro as well as in mouse models in vivo. Towards this goal we have identified about 500 disease genes. Of these, we have successfully tagged 222 murine ES and 213 HeLa cells, as well as 25 human neural stem (NS) cell lines. The protein interactors are determined of 126 of these lines by AP-MS and put into pathways using various in silico modelling approaches. Novel interactors are currently validated by iterated tagging and AP-MS analysis. The use of a fluorescence GFP-tag allows cellular localisation and tracking of protein dynamics in living cells. So far, we have imaged 159 of the genes to determine subcellular localisation. For 15 candidates mouse models have been established for functional validation of protein interactions of diseased cell types in vivo. Novel protein networks of disease genes will be presented.

DiGtoP has established novel proteomic and genetic tools and methods for functional discovery of protein networks, coupled with imaging and bioinformatics' evaluation methods. Based on this approach we are establishing a novel mammalian proteomic database relevant to disease pathways and a venue for the discovery of novel candidate genes amenable to therapeutic interventions.

Sestrin-2, a novel drug target for COPD, is a redox-sensitive repressor of PDGFRß

Presenting Author: Frank Schnütgen

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Sestrin-2 (Sesn2) belongs to a family of highly conserved antioxidant proteins with still poorly understood functions. Recently we have shown that mutational inactivation of Sesn2 in mice prevents the development of cigarette smoke-induced pulmonary emphysema by inducing the expression of PDGFRß. To investigate the possibility that Sesn2 might be a transcriptional repressor of PDGFRß, we performed protein localization and protein-protein interaction studies using mESCs and HeLa cells expressing EGFP-tagged Sesn2 under physiological conditions. While Sesn2 localized mainly to the cytoplasm in both cell types, treatment of HeLa- but not ES cells with PDGF-BB or hydrogen peroxide induced a nuclear translocation of the protein. Because unlike ES cells, HeLa cells express high levels of PDGFRß, PDGFRß signaling is presumably required for nuclear translocation. The suspected nuclear function of Sesn2 was further supported by AP/MS protein-protein interaction studies identifying several nuclear Sesn2 interaction partners. In summary, we believe that Sesn2 is part of a PDGFRß repressor complex that controls PDGFRß expression in a redox dependent manner. In collaboration with our DiGtoP partners, we will dissect this complex and map its binding site within the PDGFRß promoter region using chromatin immunoprecipitation (ChIPseq).

Mutations in Kctd1 and Pou3f3 in mice are associated with behavioral and neurological impairments

Presenting Author: Lore Becker

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Two ENU-derived mutant mouse lines characterized by elevated plasma urea levels were analyzed by systematic phenotyping in the primary screen of the German Mouse Clinic (GMC). Here we present the data from behavioral and neurological analysis of these urea high mouse lines with different genes and thus different mechanisms involved.
HST014 harbors a mutation in the kctd1 gene (potassium channel tetramerisation domain containing 1). This gene shows brain expression during development and the gene product Kctd1 may function as a transcriptional repressor by inhibiting the transactivation of the AP-2 family. No mutant was described for this gene before. Since homozygous animals are lethal early postnatal, heterozygous animals were analyzed. HST014 mice show increased prepulse inhibition (PPI), less transfer arousal and tail elevation as well as decreased locomotor activity.
In HST011 is a recessive mutation of pou3f (POU class 3 homeobox 3), a transcription factor expressed in CNS and kidney during development. Published knockouts showed neonatal mortality and developmental defects in the forebrain. Our mutants are viable and could be analyzed for neurological alterations in adult animals. HST011 mice show several behavioral and neurological alterations. In the Open field, mutant mice showed increased locomotor activity and decreased rearing by the mutant mice as well as increased centre time by the mutant mice. Acoustic startle reactivity and prepulse inhibition was decreased. During a SHIRPA protocol several parameters were changed and mutant mice showed head tossing and a reduction of grip strength. Rotarod performance was rather improved and there was a reduction of auditory brainstem response (ABR).
These are the first analyses of viable mouse mutants of the respective genes and suggest that the mutations are responsible for behavioral and neurological dysfunctions in these mice.

EMMA - The European Mouse Mutant Archive

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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. At present EMMA holds more than 3300 mouse mutant strains.

The EMMA network is comprised of 14 partners from 11 different countries who operate as the primary mouse repository in Europe. EMMA is funded by the partner institutions, national research programmes and by 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 cryopreservation courses to promote the use and dissemination of frozen embryos and spermatozoa. Dissemination of knowledge is further fostered by a dedicated resource database. EMMA's technology development programme focuses on improving sperm cryopreservation methods, the implementation of laser-assisted IVF and ICSI protocols.

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.

Data mining of the German Mouse Clinic expression profile compendium

Presenting Author: Marion Horsch

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The German Mouse Clinic (GMC) systematically analyses genetically modified mouse models (MMLs) including the molecular phenotyping screen for transcriptome analysis. Organs for expression profiling analysis are selected based on conspicuous phenotypes in other GMC screens or on known or predicted mutant phenotypes and gene functions. During the last 10 years one of the largest expression profiling dataset was generated for mouse models. In total, 238 organs (including brain, liver, spleen, thymus, kidney, skeletal muscle, heart, testis, lung and pancreas) of 125 MMLs were analysed. First, we compared expression patterns of control animals originating from different mouse source using Hierarchical cluster analysis (HCL). As expected, higher similarities between the control mice of the same genetic background were detected, however, group effects were also observed possibly caused by mixed background, number of backcrosses or housing conditions. Second, statistical methods (multiclass analyses for identification of regulated genes) were used to identify co-regulated genes between the different MMLs. The results indicate a tendency of higher similarities of gene expression patterns between MMLs on the same genetic background. Additionally, groups of co-regulated genes were observed in brain, heart, muscle, spleen and thymus in two to four MMLs. Next steps will be the functional analysis of the identified groups of genes to determine affected pathways and the more detailed analysis of the role of the genetic background. Further, we will enlarge the metadata analysis by a comparison of transcriptome profiles with other 'classical' phenotyping data generated in the GMC expecting similarities also between these parameters.

Phenotypic characterization of viable ENU-induced Pou3f3 and Kctd1 mouse mutants: Renal function, clinical-chemical, hematological and endocrine findings

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Two ENU-induced mutant mouse lines, HST011 and HST014 with an inherited phenotype characterized by elevated plasma urea levels were submitted to primary GMC phenotyping. Here we present new insights into the function of the mutated genes based on the genetic and phenotypic characterization of these mouse lines:
Genetic characterization of mouse line HST011 revealed a mutation in Pou3f3, a transcription factor expressed in neural tissues and kidneys during embryonic development. Homozygous knockout mice for this gene showed neonatal mortality. One day old homozygous knockout mice have been shown to have increased plasma urea and K levels with renal hypoplasia,delopmental defects in the forebrain and the loop of Henle. In mouse line HST014, we identified a mutation in Kctd1, coding for KCTD1, a protein expressed in the mammary gland, kidney, brain, and ovary. The biological function of KCTD proteins remained unclear so far.
Mutant animals of both lines show mild to moderate polyuria, calciuria and decreased uric acid excretion as indicators of impaired renal function. In plasma we found uremia and hyperkalemia, increased alpha-amylase activities as well as decreased protein and corticosterone concentrations in both lines. In homozygous mutant HST011 mice also parameters associated with bone and energy metabolism (ALP, lipid and glucose values) were significantly affected by the genotype. Increased chloride and decreased lactate levels suggested changes in acid-base balance. In heterozygous HST014 mutants chloride levels were significantly decreased, suggesting alkalosis in these animals.
Mutants of both lines showed erythropenic anemia, and HST011 mutants additionally decreased platelet counts and slightly increased white blood cell counts.
These are the first analyses of viable mouse mutants of the respective genes and suggest that renal function, acid-base balance, steroid metabolism and hematopoiesis are affected by the mutations in these genes.

Severe Combined Immunodeficiency and Granulocytosis Caused by a Point Mutation in an Immunoproteasome Subunit

Presenting Author: Irina Treise

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The German Mouse Clinic performs highly standardized systemic phenotyping of mutant mouse lines as model systems for human diseases. The pathophysiology of many diseases crucially involves cells and effector molecules of the immune system. Therefore, phenotypical screening for immunodeficiencies is indispensable for identification of novel genes or gene functions. In the Immunology Screen of the German Mouse Clinic a mutant mouse line, TUB006, was identified, with severe combined immunodeficiency (SCID) and simultaneously drastic granulocyte infiltrations in several organs. The mutation affects an immunoproteasome-related gene. Interestingly, point mutations in the immunoproteasome subunits are also known to be linked to autoinflammatory diseases in humans. Here, we present the results of phenotypic analyses of the TUB006 mouse line.

Assessing quality of next generation sequencing alignment data with Qualimap

Presenting Author: Konstantin Okonechnikov

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Next Generation Sequencing (NGS) is an important and widely-used discovery instrument in modern genomics, which allows to investigate biological processes at an unprecedented level. However, NGS data is prone to errors due to specifics of the technology. Thus quality control and detection of possible biases are required for reliable data interpretation.
A number of tools for NGS data quality assessment were developed recently. Some of them, such as FastQC[1], are designed to directly analyze the raw reads output of the sequencer. Other programs like RNA-SeQC[2] are designed for a specific sequencing approach and are not suitable for general alignment analysis. Finally, tools like SAM Picard[3] allow to compute various alignment metrics by means of individual scripts, lacking a common pipeline.
He we present Qualimap, a tool for NGS data quality assessment[4]. Qualimap provides an overview of the alignment data that helps to detect biases and eases decision-making for further analysis. It offers three pipelines: BAM QC, RNA-seq QC and Counts QC. Each pipeline produces an easy-to-interpret report with a descriptive summary and a number of plots. BAM QC calculates general alignment metrics like mapping statistics or homopolymer indel distribution, along with particular aspects of the alignment such as genome fraction coverage or duplication rate. Conveniently, BAM QC can be region-based and thus is applicable for any type of sequencing data. RNA-seq QC is designed for transcriptome sequencing data. It provides gene coverage plots and 5'-3' bias estimation. Counts QC can also be applied for RNA-seq data, but accepts any general feature counts. It computes global saturation, samples correlation and compares counts in various feature groups.

[1] http://www.bioinformatics.babraham.ac.uk/projects/fastqc
[2] Deluca, et al, (2012) Bioinformatics (2012) 28 (11): 1530-1532
[3] http://picard.sourceforge.net
[4] Garcia-Alcalde et.al, Bioinformatics (2012) 28(20): 2678-2679

RNA-Seq Analysis PipeLine (RAPL) – A computational tool for the analysis of deep-sequencing based transcriptome data

Presenting Author: Konrad U. Förstner

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Transcription represents an essential layer between the blueprint (DNA) and the structural, regulatory and catalytic components of the cell (proteins as well as RNAs). RNA molecules do not only act as messengers that carry the information for the synthesis of proteins or as the structural components of the protein biosynthesis machinery, but can also carry out regulatory and catalytic functions in the cell themselves. Thus, the quantitative and comparative analysis of transcripts is an important tool for the understanding of genes expression changes as well as the underlying regulatory networks.
RNA-Seq - the examination of RNA species by massively parallel sequencing technologies - is a potent way to perform such transcriptome analyses at single-nucleotide-resolution and with a high dynamic range. In order to extract information from the raw RNA-Seq data, several steps, which in part can be computationally intensive, have to be conducted. RAPL (RNA-Seq Analysis PipeLine) covers the crucial steps of such analyses and combines them into an easy-to-use tool with a consistent command line interface. It performs clipping and filtering of raw cDNA reads, mapping to reference sequences, coverage calculation, gene based quantification and comparison of expression levels. Moreover, it provides several statistics on the mapping efficiency and generates files for visualization of the results in a genome browser. The behavior of RAPL is highly configurable in order to adapt it to the specific needs of the user. To leverage the full power of modern computers, most parts of RAPL offer parallel data processing. We have successfully used RAPL for the analyses of whole transcriptome data from pro- and eukaryotes, RNAs isolated from co-immunoprecipitation experiments as well as other subclasses of RNA species from the cell. RAPL will be publicly available under an open source license in the near future.

Analyzing Illumina gene expression microarray data from different tissues: Methodological aspects of data analysis in the MetaXpress consortium

Presenting Author: Claudia Schurmann

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Microarray profiling of gene expression is widely applied in molecular biology and functional genomics. Experimental and technical variations make meta-analysis of different studies challenging. Within the MetaXpress consortium consisting of three German population-based cohorts (SHIP-TREND, KORA F4 and GHS) our aim was to quantify the effects of technical and biological factors on gene expression data and to reduce variability due to sample processing.

In 3358 samples we analyzed gene expression profiles derived from whole blood cells and monocytes using the Illumina HumanHT-12 v3 BeadChip array. We performed a principal component analysis on the probes' intensities and applied the EigenR2 algorithm to identify

the amount of variance explained by pre-selected variables. The possible impact of these variables was tested by associating the expression levels with BMI and a randomly generated normal distributed phenotype. Possible negative hybridization effects due to SNPs located within probes were analyzed by testing the association of 2148 probes and their enclosed SNPs.

We identified few but important technical factors that should be used for adjustment in gene expression analyses to improve reliability of the results without negatively affecting potential true positive associations. The confounding of biological parameters was remarkably small compared to those of technical factors. Although most SNPs within probes were associated with decreased expression signal intensity per mismatch allele, 45% were associated with increased signal intensity. This shows that SNPs do not have a consistent effect on hybridization efficiency: It is therefore not necessary to exclude SNP-containing probes from association analyses.

We will use our standardized data preprocessing and analysis platform to further facilitate meta-analyses of gene expression studies and to explore the expression profile of blood cells associated with several clinical parameters.

National Genome Research Network

Poster Presentation Abstracts

Symposium VI

Personalized Medicine

Effect of Brain Structure, Brain Function, and Brain Connectivity on Relapse in Alcohol-Dependent Patients

Presenting Author: Anne Beck

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Introduction: In alcohol-dependent patients brain atrophy and cue-induced brain response may predict relapse. However, to date the interaction between both factors has not been studied. Here, we assessed whether results from structural and functional magnetic resonance imaging (MRI/fMRI) are associated with relapse in alcohol-dependent patients.
Methods: 46 detoxified alcohol-dependent patients and 46 age and gender-matched healthy subjects participated in a cue-reactivity paradigm (using alcohol-associated and neutral stimuli). Patient group was sub-divided post-hoc regarding relapse within 3 months after scanning (16 abstainers, 30 relapsers). We assessed local gray matter volume, fMRI activation, joint analyses of structural and functional data with Biological Parametric Mapping and connectivity analyses adopting the psycho-physiological interaction (PPI) approach.
Results: Subsequent relapsers showed pronounced atrophy in bilateral orbitofrontal cortex (OFC) as well as right medial prefrontal (MPFC) and anterior cingulate cortex compared with healthy controls and abstainers. The local gray matter volume corrected cue-induced brain response in left MPFC was enhanced for subsequent relapsers, while abstainers displayed an increased neural response in midbrain and ventral striatum. The PPI analyses showed a stronger functional connectivity between midbrain and left amygdala as well as left OFC for alcohol-associated compared to neutral stimuli in abstainers compared to relapsers.
Conclusions: Subsequent relapsers displayed increased brain atrophy in brain areas associated with error monitoring and behavioral control. Correcting for gray matter reductions, in these patients alcohol-related cues elicited increased activation in brain areas associated with attentional bias towards these cues, while in patients who remained abstinent, increased activation and connectivity was observed in brain areas associated with processing of salient or aversive stimuli.

Neuronal activation during processing of aversive faces predicts treatment outcome in alcoholism

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Neuropsychological studies reported decoding deficits of emotional facial expressions in alcohol-dependent patients, and imaging studies revealed reduced prefrontal and limbic activation during emotional face processing. However, it remains unclear whether this reduced functional activation is mediated by alcohol-associated volume loss and whether it interacts with treatment outcome.
In the present study, we combined analyses of neuronal activation during an aversive face-cue-comparison-task and local grey matter volumes (GM) using Biological Parametric Mapping in 33 detoxified alcohol-dependent patients and 33 matched healthy controls.
Alcohol-dependent patients displayed reduced activation towards aversive faces-neutral shapes in bilateral fusiform gyrus (BA18/19), right middle frontal gyrus (BA46/47), right inferior parietal gyrus (BA7) and left cerebellum compared to controls, which were explained by GM differences (except for cerebellum). Enhanced functional activation in patients versus controls was found in left rostral ACC and MPFC (BA10/11), even after GM reduction control. Reduced amygdala activation correlated with more abstinence days and less binge drinking days during six-month follow-up, while increased ACC activation correlated significantly with less (previous) lifetime alcohol intake, longer abstinence and less subsequent binge drinking in patients. Right ACC GM loss in patients was associated with shorter abstinence and more binge drinking during follow-up. High lifetime alcohol intake appears to impair treatment outcome via its neurotoxicity on ACC integrity.
Low atrophy and high activation of the affective ACC elicited by affective faces thus appear to be resilience factors predicting better treatment outcome. Therapeutic interventions (e.g. training of emotion evaluation) may enable patients to cope with social stress and to decrease binge drinking/relapses after detoxification.

Evaluation of amplicon-based semiconductor sequencing for diagnostic screening of formalin-fixed paraffin embedded tumor material

Presenting Author: Volker Endris

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In view of a personalized medicine, the screening of somatic mutations in an individual tumor that predict therapeutic outcome has become an increasing need in the clinical and molecular diagnostics. In most laboratories, Sanger sequencing has become an accepted standard, but due to the limited screening throughput and the increasing demand for targeted sequencing, the application of next-generation sequencing in the molecular diagnostic has turned into focus. Whole-genome or whole-exome sequencing approaches provide a comprehensive view of an individual tumor mutation load, but the current high costs and the excess of information without a direct clinical implication limit the routine use of this technology. In this study, we evaluated a targeted resequencing approach that focuses on somatic hotspot cancer mutations based on semiconductor sequencing. We especially took attention on the reliability of the method concerning sample quality, mutation type and reproducibility. We used DNA extracted from formalin-fixed, paraffin embedded tumor tissues (biopsy and resection) of different tumor degree and overall quality, that were previously screened for the presence of EGFR mutations by Sanger sequencing. 190 amplicons covering hotspot mutations in 46 genes were generated in a multiplex PCR reaction and 8 samples were sequenced on a single IonTorrent 318 chip. We were able to successfully sequence all samples with a mean coverage rate of 670.000 reads (AQ20:560.000) and an average read depth of 2947 AQ20 reads / amplicon. In all but one sample, variant calling identified the EGFR mutation, missing only one low level 9bp insertion in exon 20. Moreover, we identified additional 43 mutation in 17 genes and uncovered three previously unknown EGFR amplifications. Taken together, amplicon-based semiconductor sequencing is a powerful and cost-effective method working with low-quality DNA material, enabling routine diagnostic next-generation sequencing.

Analysis of signal transduction pathways upon EGFR inhibition in colorectal cancer cell lines

Presenting Author: Holger Armbruster

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It is well known that KRAS mutations in colorectal cancer are associated with the patients´ response to therapy with the monoclonal antibodies Cetuximab or Panitumumab, inhibiting the EGF receptor. In contrast, small molecule drugs like Erlotinib or Gefitinib, which are directed against intracellular phosphorylation sites of the EGF receptor, are currently no therapy options in colorectal cancer.
To understand the molecular impact of these small molecule drugs onto the activity of signal transduction pathways in cancer cells, we performed an in vitro analysis of Erlotinib and Gefitinib sensitivity in eight colorectal cancer cell lines. A first screening revealed up to five fold differences in drug sensitivity between the cells. Based on these results, five cell lines (GEO, CaCo2, LIM1215, Colo678 and HT29) were selected for a mutation analysis using the MassARRAY technology based OncoCarta Panel v1.0 covering 238 somatic mutations across 19 common oncogenes. No association between mutational status and drug sensitivity was found. The effects of Erlotinib and Gefitinib on the phosphorylation status of twelve key signaling proteins downstream of the EGFR were analyzed using the Luminex xMAP technology. As expected, the effects of Erlotinib and Gefitinib were very similar. We found evidence for the NF-κB, P53 and HSP27 pathways, to be associated with drug sensitivity. In contrast, inhibition of the MAPK pathway, which is the major effector pathway of EGFR action, was only observed in CaCo2 and Colo678 cells and was not associated with drug sensitivity. In summary, the inhibition of the EGFR has significantly different effects on various colorectal cancer cell lines. These differences have to be further investigated in order to understand the molecular diversity of inhibitory effects of Erlotinib and Gefitinib application in these cellular models.

GRHL1 inhibits tumorigenicity and is a prognostic marker in neuroblastoma

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Inhibition of histone deacetylase activity by small molecules favorably impacts neuroblastoma biology in preclinical models. Here we aimed to decipher the underlying molecular mechanisms that have remained enigmatic. GRHL1 is a highly conserved neuroectodermal developmental transcription factor with no prior defined role in oncology. In a genome-wide expression profiling, GRHL1 was among the five strongest immediate early response genes to histone deacetylase inhibitor (HDACi) treatment in an in vitro differentiation model in a time-course. Different HDACi, including those clinically approved, similarly induced GRHL1 expression in a panel of cell lines as well as neuroblastoma xenografts in mice, supporting GRHL1 induction as a common event of HDAC inhibition in neuroblastoma cells. High GRHL1 expression in 380 neuroblastomas was prognostic for favorable event-free and overall patient survival and significantly correlated with localized disease stage, young age, favorable Shimada/INPC histology, MYCN single copy and 1p wildtype status, and a favorable PAM transcriptional profile. These findings were confirmed in two independent cohorts of 102 and 88 neuroblastomas. Immunohistochemistry revealed strong nuclear GRHL1 expression in favorable, but no expression in unfavorable tumors. Enforced GRHL1 expression in cell lines strongly suppressed anchorage-independent colony formation in soft agar, and distinctly attenuated xenograft growth in mice, suggesting that GRHL1 targets tumorigenic pathways.
In summary, GRHL1 is more active in favorably prognostic, differentiated tumors than in poor prognostic, aggressive tumors. Functional relevance is provided by the strong tumor-suppressive effects caused by GRHL1 in different preclinical neuroblastoma models. From a therapeutic perspective, triggering GRHL1 expression by pharmacological inhibition of HDAC activity may tip the scales in favor of a favorable phenotype.

From iCHIP to big data - new ways to handle complexity launched by NGFN

Presenting Author: Chris Lawerenz

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From the beginning of NGFN onwards we have organized data for different networks. Within these 12 years the requirements needed to sustain service structures have increased substantially with regards to expenses and complexity.

The data management system iCHIP (www.ichip.de) has been enhanced as the central database for different consortia. Over the NGFN time span we have managed the data of divers NGFN projects as ENGINE, Neuroblastoma, CancerNet, NeuroNet, Brain Tumor Net, Cardiovascular, Infection/Inflammation, PaCaNet, SMP Bioinformatics, SMP RNAi, SMP Cell and SMP cDNA. Firstly, we covered two dimensional experiments in the field of transcriptomics. Subsequently, we included e.g., proteomics data and RNAi microscopy images. Next-generation sequencing (NGS) has now superseded the microarray approach as the main technology for genome analysis.

The quantity of this new kind of data on a petabyte level does not only correlate to the complexity of data. The underlying processing steps from original raw data to meaningful contents get more and more extensive. To reduce the enormous costs of data processing in terms of man power and time, we have developed a novel, fully automated job control pipeline called One Touch Pipeline (OTP). Currently, OTP functionality encompasses all relevant steps for the final analysis. Quality Controlling (QC), based on e.g. fastqc and complex alignment methods are also included. All sequencing runs of our projects are now monitored. This new way of organization enables the appropriate processing of all sequence data for reputable projects. To cite some examples: NGFN, the German ICGC projects PedBrain, MMML and Early Onset Prostate Cancer, the “Deutsches Epigenom Programm” (DEEP) as part of the International Human Epigenome Consortium (IHEC), the Heidelberg Initiative for Personalized Medicine (HIPO).

The backing of NGFN has been fundamental for the construction of the professional and mature data centre at DKFZ.

Moving PacaNet-Biobanking into the NGS-age

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The sophisticated nature of research-driven clinical and surgical practices demands comprehensive organization of process and data flow. Within NGFN-project, PacaNet, the European Pancreas Center (EPZ) and the team of Roland Eils at the DKFZ have generated a defined set of computer-driven measures to organize essential services supporting data-driven high quality translational research and development of the personalized treatment options for patients affected by pancreatic cancer. The research bio-database, PancoBank, utilizes DKFZ iCHIP (www.iCHIP.de) technology linking the collection of biopsies and biofluids with clinico-pathological data comprising results of genome-wide screenings in order to elucidate pancreatic pathogenesis and to develop molecular signatures enabling stratification of the patients for novel treatments. The PancoBank integrates two units. The first unit is an Amazon-like platform implementing web-based registration, management of clinical specifications, specimen quality assessment, and distribution of biospecimens according to the SOPs. It allows remote searches of the database by collaboration partners and provides them with differentially stained images of collected tissues, and thus information about cellular composition of samples — one of the most decisive aspects in the interpretation of the HTS data. The second unit facilitates upload and linkage of time-resolved pseudonymized clinico-pathological EPZ records to external HTS data provided by collaborating consortia. The data include images of mRNA, miRNA, SNP, protein and antibody profiles, and Next Generation Sequencing (NGS) data.

MYCN and HDAC2 cooperate to repress miR-183 signaling in neuroblastoma

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MYCN is a master regulator controlling many processes necessary for tumor cell survival. Inhibition of HDAC activity suppresses malignant properties of MYCN-amplified neuroblastoma cells. Here, we unravel a micro-RNA network, at least, partially causing these tumor-suppressive effects.

Expression changes from pan-HDACi treatment were analyzed by miRNA profiling. Of the 1000 miRNAs, pan-HDACi treatment most strongly induced miR-183 expression. Up-regulation reached 80-fold in a time-course using qRT-PCR analysis, validating microarray results. Similar effects were observed in vitro in 4 neuroblastoma cell lines as well as in neuroblastoma xenografts. Enforced miR-183 expression induced apoptosis and inhibited anchorage-independent colony formation of MYCN-amplified cells in vitro and strongly reduced xenograft growth in mice. Experiments to identify the HDAC(s) involved in miR-183 transcriptional regulation showed that HDAC2 depletion induced miR-183. Conversely, HDAC2 overexpression reduced endogenous miR-183 and counteracted the induction caused by HDAC2 depletion. ChIP revealed that HDAC2 was recruited to the miR-183 promoter, and that HDAC2 depletion enhanced miR-183 promoter-associated histone H4 pan-acetylation, suggesting epigenetic changes preceded transcriptional activation. Because H3K27 trimethylation at the miR-183 promoter was higher and endogenous miR-183 expression was up to 15-fold lower in MYCN-amplified cells, we tested whether MYCN recruits HDAC2 to the miR-183 promoter, and detected MYCN and HDAC2 in the same complexes at this site by Re-ChIP. Further, we demonstrated that MYCN depletion reduced recruitment of HDAC2 and increased miR-183 expression.

These data reveal the tumor suppressive properties of miR-183 in neuroblastoma that are jointly repressed by MYCN and HDAC2, and suggest a possible novel way to bypass MYCN in treating patients with MYCN-amplified tumors.

HDAC11 controls mitotic cell cycle progression of neuroblastoma cells

Presenting Author: Theresa M. Thole

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Expression of HDAC11, the most recently identified histone deacetylase, is restricted to the cell nuclei in poorly differentiated neuroblastomas. We have previously shown that HDAC11 depletion induces a prognostically favorable neuroblastoma transcriptome, partly by reverting BMP4 epigenetic silencing, thereby, triggering this developmental pathway. Here we aimed to decipher the functional relevance of further distinct alterations in gene expression caused by HDAC11 depletion.

Whole-genome expression was evaluated in p53-wildtype and -mutant MYCN-amplified neuroblastoma cells following HDAC11 depletion. Differential expression of candidate genes in primary neuroblastomas was assessed in three independent datasets from 468, 102 and 88 tumors.

HDAC11 depletion caused the genome-wide differential expression of 259 and 167 genes in p53-mutant BE(2)-C and p53-wildtype IMR-32 cells, respectively. The biological functions of genes consistently regulated over time across each cell system were assessed by analyzing gene ontology term over-representation. Genes necessary for mitotic cell cycle progression and cell division were most prominently enriched. All ten of these genes were strongly repressed by HDAC11 depletion, followed by a G2/M arrest and apoptosis in functional assays. High candidate gene expression levels in primary neuroblastomas strongly correlated with unfavorable overall patient survival in all 3 datasets, demonstrating their clinical relevance. Depletion of 6 candidate genes, singly, reduced metabolic activity up to 90% and increased caspase-3/7-like activity up to 10-fold, mimicking the phenotype caused by HDAC11 depletion.

Here we investigate a group of cell cycle-promoting genes repressed by HDAC11 depletion, being both, predictors of patient outcome and essential for neuroblastoma cell viability. Our data further support HDAC11 inhibition as a novel targeted therapeutic approach for the treatment of high-risk neuroblastomas, regardless of p53 status.

The epidermal growth factor receptor is essential for Kras-driven pancreatic carcinogenesis

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Introduction: Constitutively activating mutations in the Kras gene are found in up to 90 % of all pancreatic ductal adenocarcinoma (PDAC) patients and are sufficient to drive PDAC initiation and progression in the well-established pancreas-specific endogenous KrasG12D mouse model, although it needs several weeks or additional pancreatitis induction. Epidermal growth factor receptor (EGFR) is the only approved target for targeted PDAC therapy. Despite its elevation in PDAC this is very peculiar since EGFR is upstream in the Ras signaling pathway and therefore should be redundant in Kras mutant cancers. To investigate this paradox in detail we examined the effect of EGFR ablation in vivo in the background of the KrasG12D mouse model.

Experimental design: Applying the Cre/lox system Egfr was deleted and KrasG12D activated in PTF1A-positve pancreatic progenitor cells. As additional carcinogenic stimulus acute pancreatitis was induced using cerulein. Inhibitor studies were applied to investigate the role of EGFR and ERK in PDAC development and progression. Acinar to ductal metaplasia (ADM) was closely examined applying different in vivo and in vitro approaches. Cell line studies in murine EGFR-KO deficient PDAC cells gave insight into ERK and RAS activation levels.

Results and Conclusion: KRAS-driven pancreatic tumorigenesis but not progression in vivo and in vitro strongly depends on EGFR. EGFR activity is necessary for the formation of transformation-sensitive metaplastic ducts through maintaining high ERK activation levels. ADM, even in the context of pancreatitis-induced PanIN formation in KrasG12D mice, is blocked in EGFR-KO pancreata, and also upon MEK inhibition. Additional p53 deletion circumvents EGFR dependency. These results allow new insight into the molecular basics of cancer formation and might change the thinking of linear pathway signaling.

Identification of drug-associated proteins in NSCLC xenograft models by reverse-phase-protein-arrays

Presenting Author: Helen Hülsmann

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At present, most non-small cell lung cancer (NSCLC) treatments are not adapted to the individual response of a patient. The stratification of patients for the most efficient response to conventional chemotherapeutics and targeted therapies will improve established therapy schemes and patient's perspectives. Our project aims at unraveling the influence of specific signaling molecules on the response to common NSCLC drugs as conventional chemotherapy and different novel EGFR-inhibitors. We searched for predictive markers for the response of NSCLC tumors to certain drugs and novel combinations of treatments, which may be useful for therapy decisions towards a more efficient personalized treatment.
We used the reverse-phase protein array technology (RPPA) to quantify the expression of 107 proteins of cancer-relevant pathways (e.g. MAPK, JAK/STAT, PI3K/AKT) in 64 patient derived NSCLC xenograft models. The tumor models are characterized by different response rates upon treatment with established chemotherapeutics (e.g. Gemcitabine, Paclitaxel, Carboplatin) and EGFR-targeted therapies (Cetuximab, Erlotinib). Statistical analysis indicated significant associations between the expression of distinct proteins before treatment and the response to certain drugs. Proteins of the ErbB signalling pathway were differentially expressed in Carboplatin responders and non-responders. MEK1 upregulation was observed upon Cetuximab treatment in responders. We revealed an association between higher Phospho-SRC expression and increased Gemcitabine response rates. Based on RPPA analyses candidate targets were selected for validation experiments. Predictive targets for drug sensitivity are being analyzed to investigate the dependency between target protein activity, downstream signaling and response to distinct drugs in tumor cell lines and xenograft models. Furthermore, we suggest novel potent combinations of inhibitors/activators of predictive targets and established drugs.

Serum miRNAs associated with early relapse in operable lung adenocarcinoma patients

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microRNAs (miRNA) are short non-coding RNAs that emerge as a new class of markers for diagnosis and prognosis in non-small-cell lung cancer (NSCLC). miRNAs cannot only be stably quantified in tissues but as well in body fluids like serum. However, little is known about circulating miRNAs as prognostic markers in lung adenocarcinoma (AdC) patients.
The aim of this study was to identify novel miRNAs in serum and tissue as prognostic markers involved in disease recurrence of patients with early-stage lung AdC.
Serum miRNAs were screened using qRT-PCR based arrays comparing adenocarcinoma patients (n= 40) with and without recurrence within the time period of 24 months after surgery. Selected miRNAs associated with disease recurrence were validated in the sera of an independent patient cohort (n= 114), compared with sera of patients with advanced AdC and benign diagnosis, and further analysed in 46 fresh-frozen matched lung AdC tissues.
Two circulating miRNAs were identified in the screening and confirmed in the validation cohort to be increased in sera of early-stage lung AdC patients suffering from recurrence within two years. The differentiation between lung AdC patients with low and high risk for recurrence was improved by accounting for both the identified miRNA and tumour stage. The expression of this miRNA in NSCLC tissues did not reveal an association with metastatic spread as was observed for the circulating form in the sera of early-stage lung AdC patients. Currently, we aim to determine the expression of specific miRNAs in formalin-fixed, paraffin embedded (FFPE) tissue of NSCLC patients using miRNA in-situ hybridization.
In conclusion, circulating miRNAs were found to be associated with a high risk of recurrence in early-stage lung AdC patients and may serve as putative non-invasive prognostic markers.

Whole genome and whole exome sequencing of gastric cancer samples

Presenting Author: Daniela Esser

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Gastric cancer causes 10% of the cancer deaths worldwide. Improved understanding of the biology of cancer is required to improve patient outcomes. To further analyze the genetic basis of gastric cancer, we sequenced the whole genome of a microsatellite stable and a microsatellite unstable gastric carcinoma and the genome of the matched normal tissue samples on the Illumina HiSeq with an average coverage of 49x. In order to increase the read depth in the coding regions we performed whole exome capture (Agilent) followed by sequencing on the Solid 4 to an average coverage of 55x.
We analyzed single nucleotide variants (SNVs) and structural variants (SVs) like insertions, deletions, translocations, inversions and tandem duplications. All variants occurring in the normal samples were subtracted from the tumor samples and to identify somatic mutations, all variants found in the dbSNP or 1000 Genomes Project databases were excluded. After an additional quality check the results were compared with common databases like OMIM, HGMD and GWAS in order to find known cancer associated mutations. The main SNV and small indel candidates were selected by quality, genomic region, damaging prediction (Sift, PolyPhen), base conservation (PhyloP) and gene conservation. We performed pathway (KEGG) and functional (GO) analysis of the remaining SNV and SVs using 1087 whole genomes from the 1000 Genomes project as a normal SNV matrix.
Using this comprehensive strategy we were able to identify a multitude of novel potentially damaging mutations that are followed up by functional assays. Recurrency is assessed in an independent cohort of gastric cancer patients.
The results highlight a strategy of an exemplary tumour genome analysis that combines both exome and whole genome sequence information with two different NGS platforms, uses population-based whole genome resources as a novel pathway-based filter and integrates SNV as well as structural variant analysis.

The pibase software for clinical genotyping of single nucleotide variants and pair-wise comparisons of next-generation sequencing alignment files

Presenting Author: Michael Forster

Michael Forster (1), Peter Forster (2), Abdou Elsharawy (1), Georg Hemmrich (1), Benjamin Kreck (1), Michael Wittig (1), Ingo Thomsen(1), Björn Stade(1), Matthias Barann (1), David Ellinghaus (1), Britt-Sabina Petersen (1), Sandra May (1), Espen Melum (4,5), Markus B Schilhabel (1), Andreas Keller (6), Stefan Schreiber (1,7), Philip Rosenstiel (1), Andre Franke (1)

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Medical researchers and clinicians working with single nucleotide variant (SNV) lists, inferred by next-generation sequencing software, often need further information regarding true variants, artifacts, and sequence coverage gaps. In clinical diagnostics, for example, SNVs must usually be validated by visual inspection or several independent SNV-callers. We here demonstrate that 0.5% to 60% of relevant SNVs might not be detected due to coverage gaps, or might be misidentified. Even low error rates can overwhelm the true biological signal, especially in clinical diagnostics, in research comparing healthy with affected cells, in archaeogenetic dating, or in forensics. For these reasons we have developed a package called pibase which is applicable to diploid and haploid genome, exome, or targeted enrichment data. pibase extracts details on nucleotides from alignment (BAM) files at user-specified coordinates (e.g. via VCF input files) and identifies reproducible genotypes, if present. In test cases pibase identifies genotypes at 99.98% specificity, ten-fold better than other tools. pibase also provides pair-wise comparisons between healthy and affected cells using nucleotide signals (ten-fold more accurately than a genotype-based approach, as we show in our case study of monozygotic twins). This comparison tool also solves the problem of detecting allelic imbalance within heterozygous SNVs in copy number variation loci, or in heterogeneous tumor sequences. The pibase export formats include human-readable tables, VCF files, and phylogenetic network files.

A manuscript version of this presentation has recently been published online on 10 September 2012 in Nucleic Acids Research, 2012, 1–12 doi:10.1093/nar/gks836

Protein biomarker signature for risk classification of hormone receptor positive breast cancer patients identified by reverse phase protein array based tumor profiling

Presenting Author: Johanna Sonntag

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Around 70% of breast cancer cases belong to the luminal intrinsic molecular subtype, characterized by hormone receptor overexpression. The subtype can be further divided into luminal A and luminal B, which is commonly used as surrogate for good and bad prognosis, respectively. This classification is crucial for therapy decision as patients of the luminal B subtype are at high risk of recurrence and require chemo-endocrine therapy, contrasting low risk patients who could be spared chemotherapy. However, accurate definition of low and high risk luminal breast cancer in the routine clinical setting has remained a challenge so far.

The objective of our study was the identification of a robust protein biomarker panel to facilitate the risk classification of luminal breast cancer patients. To achieve this aim we have applied reverse phase protein arrays (RPPA) to quantify over 120 breast cancer relevant target proteins of hormone receptor positive breast cancer tumor samples. Subsequently, we used a novel bioinformatics workflow combining a bootstrap approach with three different classification methods for biomarker selection.

Our results confirm that markers for cell proliferation are prominent factors to distinguish between low and high risk tumors with Ki-67, TOP2A, and PCNA appearing among the top hits. However, NDKA, RPS6, and caveolin 1 were selected as prime candidates. Comparably to Ki-67, NDKA and RPS6 were expressed at an elevated level in high risk tumors whereas caveolin 1 was observed to be downregulated. To validate our RPPA derived result we have applied Western blot, immunohistochemistry, and mRNA profiling.

In summary, we have identified a protein biomarker signature (consisting of caveolin 1, NDKA, RPS6, and Ki-67) using RPPA based tumor profiling which facilitates the risk of recurrence classification in luminal breast cancer. In addition, we present RPPA as promising experimental platform for the identification of biomarkers in clinical samples.

The Virtual Patient - The IT Future of Medicine

Presenting Author: Ralf Sudbrak

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The IT Future of Medicine (ITFoM) initiative will produce computational models of individuals to enable the prediction of their future health risks, progression of diseases and selection and efficacy of treatments while minimizing side effects. As one of six Future and Emerging Technologies (FET) Flagship Pilot Projects funded by the EC, ITFoM will foster the integration of technology development in functional genomics and computer technologies to enable the generation of patient models to make them available for clinical application. The realization of the patient model is based on the recent breakthroughs in sequencing technology that enables the high-throughput analysis of a large number of individual genomes and transcriptomes. The genome profile will be integrated with proteome and metabolome information generated via new powerful chromatography, mass spectrometry and nuclear magnetic resonance techniques. Computational and mathematical tools enable the development of systems approaches for deciphering the functional and regulatory networks underlying the complex biological systems and form the basis for the future patient model.
The –omics information will provide the basis to establish integrated molecular, physiological and anatomical models of every individual in the health care system. The first approach to the “Virtual Patient” modeling system that has been generated at the Max Planck Institute for Molecular Genetics combines general information available about cancer relevant pathways with the individual tumor/patient information. This individualized model will not only be able to analyze the current situation, but will allow the prediction of the response of the patient to different therapy options or intolerance for certain drugs.
ITFoM is an initiative of more than 150 academic and industrial partners from 31 countries to set up a research concept for the development of the “virtual patient”.

Public Health Evaluation Program: Promoting cancer prevention in rural communities through partnerships

Presenting Author: Samuel George Atiemo

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Public Health evaluation is recognized as being a significant part of the solution for providing better healthcare outcomes. However, there are multiple challenges to its adoption healthcare stakeholders. The challenges are not technological, but rather involve the development of the appropriate business model, such that all stakeholders can have the necessary incentives to justify adoption.

From brief presentation, multiple questions, some brief exercises and group discussions, participants will be encouraged to reflect on; the impact of public health care evaluations on the challenges of Cancer prevention and quality health care. The paper demonstrate how the evaluation of existing public health data can be used to create meaningful indicator measures to further understanding of cancer prevention related problems. We have further discussed principal criteria for and methods of implementing systems that will help deal with risk factors within the communities. After considering all aspects of cancer prevention impact assessments for different alternatives, this paper evaluates and outlines the principles and guidelines for cancer prevention strategies and interventions

From the academic perspective, cancer prevention evaluations are beneficial because they provide faculty and students with the opportunity to engage in public health practice and encounter new issues that advance the science of public health through research. From the perspective of a public health department, they address priority public health concerns and build the capacity of department personnel to conduct evaluations with internal resources. A collaborative cancer prevention evaluation and health hazard assessment programs increases public health capacity by developing new approaches to public health problems and sharing the limited resources.

Differentiation of induced pluripotent stem cells toward neurons in biocompatible hydrogels

Presenting Author: Yu-Hsuan Chang

Yung-Chih Kuo (1), Yu-Hsuan Chang (1)

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Regeneration of nerve tissue is one of the most significant challenges in contemporary surgical therapy for nervous system injury. This study presents the neuronal differentiation of induced pluripotent stem (iPS) cells in hydrogels comprising alginate and poly/(γ-glutamic acid) (γ-PGA) with surface neuron growth factor (NGF). Differentiating iPS cells in NGF-grafted alginate/γ-PGA constructs were identified by immunochemical staining of anti-SSEA-1 and anti-ß III tubulin. The results revealed that the pore diameter of hydrogels increased with an increasing weight ratio of alginate to γ-PGA. The porosity slightly decreased with an increasing weight ratio of alginate to hydrogel. In addition, an increase in the weight ratio of alginate to hydrogel raised the swelling ratio. Morphological images of differentiating iPS cells in NGF-grafted alginate/γ-PGA constructs exhibited neuronal characteristics. The surface NGF enhanced the intensity of ß III tubulin and inhibited the intensity of SSEA-1 expressed by differentiating iPS cells in cultured constructs, indicating the differentiation of iPS cells toward neurons. NGF-grafted alginate/γ-PGA hydrogels can be efficient biomaterials in the production of neurons from iPS cells for animal study and preclinical trial.
Keywords: induced pluripotent stem cell; neuron

Barth Syndrome modelling with an in vitro patient-specific cell system

Presenting Author: I-Fen Cheng

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Barth syndrome (BTHS) is an X-linked disorder caused by mutations in the tafazzin gene, which lead to decreased production and altered composition of cardiolipin (CL). CL is a phospholipid and can stabilize the respiratory chain supercomplexes. The characteristics of BTHS are cardiomyopathy, skeletal myopathy, neutropenia, abnormal mitochondria and growth retardation. Currently, a murine model of BTHS has been developed, but the abnormalities of both mitochondrial and sarcomeric structure in cardiac and skeletal muscles can only be observed until they reach 8 months old and do not faithfully represent human pathophysiology. The goal of this study was to establish an in vitro disease model of BTHS with patient-specific cells. The preliminary data showed that BTHS cells displayed fragmented mitochondrial morphology while the control cells displayed elongated mitochondrial morphology. The growth rate of BTHS patient-derived cells was significantly lower than of controls. To analyse mitochondrial function we quantified mitochondrial respiration in patient cells and in healthy controls. We observed a significant decrease in the basal oxygen consumption rate (OCR). FCCP-induced uncoupling of the respiration chain revealed a dramatic decrease in the maximal respiratory capacity. Simultaneous measurement of extracellular acidification rate (ECAR) allowed us a thorough assessment of the metabolic deficiency in BTHS patients. Interestingly, using native gel electrophoresis we are able to show that the deficiencies in respiration coincide with structural defects of respiratory chain complexes. Defects in the energetic coupling of respiratory chain lead to a massive increase in the generation of reactive oxygen species (ROS) providing an explanation for the pathogenesis of the disease. Taken together, our data demonstrate that the patient-specific cell model system provides a valuable tool in studying molecular mechanisms of BTHS and in developing novel therapeutic strategies.

Circulating microRNAs in plasma as early detection markers for breast cancer

Presenting Author: Katarina Cuk

Katarina Cuk (1,2), Manuela Zucknick (3), Jörg Heil (4), Dharanija Madhavan (1,2), Sarah Schott (4), Andrey Turchinovich (1,2), Dorit Arlt (5), Michelle Rath (6), Christof Sohn (4), Axel Benner (3), Hans Junkermann (4), Andreas Schneeweiss (4,7) & Barbara Burwinkel (1,2,7)

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In recent years circulating miRNAs have attracted a great deal of attention as promising novel markers for various diseases. The aim of our study was to investigate their potential to serve as minimally invasive, early detection markers for breast cancer in blood plasma. We profiled miRNAs extracted from the plasma of early stage breast cancer patients (taken at the time-point of diagnosis) and healthy control individuals using TaqMan Low Density Arrays (TLDA). Selected candidates identified in the initial screen were further validated in an extended study cohort of 207 individuals including 127 sporadic breast cancer cases and 80 healthy controls via RT-qPCR. Four miRNAs (miR-148b, miR-376c, miR-409-3p and miR-801) were shown to be present in significantly higher amounts in the plasma of breast cancer patients. ROC curve analysis showed that the combination of only three miRNAs (miR-148b, miR-409-3p and miR-801) had an equal discriminatory power between breast cancer cases and healthy controls as all four identified miRNAs together (AUC=0.69)*. These results have been further validated on an independent cohort of 210 individuals comprising 120 malignant and 30 benign breast cancer patients as well as 60 healthy controls. In this cohort the discrimination power between malignant breast cancer patients and healthy controls was even higher (AUC=0.77). In conclusion, the identified miRNAs might be of potential use in the development of a multi-marker blood-based test to complement and improve early detection of breast cancer. Such a multi-marker blood test might for instance provide a prescreening tool, especially for younger women, to facilitate decisions about which individuals to recommend for further diagnostic tests.

* Cuk et al., Circulating microRNAs in plasma as early detection markers for breast cancer. Int J Cancer. 2012 Aug 28. doi: 10.1002/ijc.27799. [Epub ahead of print]

In depth Study on Chemical composition and Biological Activities with cancer controversy of Neplese Piper betel L.

Presenting Author: Akash Deo

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The essential oil sample leaf of Piper betel was collected in Biratnagar,Nepal and was analyzed by using the GC-MS method at the University of Alabama in Huntsville. The yield of the oil was 0.1% pale yellow color on hydro-distillation using Clevenger type apparatus. The antimicrobial activity of chavibetol-rich betel leaf oil was observed with minimum inhibitory concentrations (MIC) on Staphylococus aureus (MIC = 625 µg/mL), Bacillus cereus (MIC = 625 µg/mL), Es-cherichia coli (MIC = 625 µg/mL), Pseudomonas aerugi-nosa (MIC =625 µg/mL), Aspergillus niger (MIC = 313 µg/mL), and Candida albicans (MIC = 1250 µg/mL). In vitro cytotoxic activity is also exhibited by chavibetol-rich Piper betel leaf oil on MCF-7 human breast adenocarcinoma cells with 100% killing at 100 µg/mL.

Argonaute-specific miRNA profiles in human cells and blood plasma

Presenting Author: Andrey Turchinovich

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Studies of miRNA association with Argonaute proteins in mammalian cells have indicated lack of bias toward particular AGO. We compared the total miRNA content in AGO1 and AGO2 immunoprecipitates obtained from MCF7 adenocarcinoma cells using TaqMan Low Density miRNA Arrays and successfully verified selected miRNAs with qPCR. For most of the miRNA species AGO1 and AGO2 profiles were well correlated, however, some miRNAs demonstrated consistent biases toward one of the AGO proteins. Furthermore, miRNAs which were predominantly AGO2-associated derived mostly from sense strands of the corresponding pre-miRNAs while the majority of AGO1 biased miRNAs originated from antisense strands of the pre-miRNAs. Additionally, we show that circulating miRNA in human blood plasma can be immunoprecipitated with both AGO1 and AGO2 antibody. However, unlike in cell lysates, AGO1 and AGO2 associated miRNA profiles in plasma did not correlate, indicating that many cell types contribute to circulating miRNA (given that expression of AGO proteins is tissue specific). Furthermore, AGO-specific miRNA profiles in blood cells differed significantly from miRNAs profiles in plasma indicating that most circulating miRNAs are likely to derive from non-blood cells. Since circulating miRNAs hold great promise as biomarkers for numerous cancers and other diseases, we hypothesize that AGO-specific miRNA profiles might add an additional dimension to circulating miRNA-based diagnostics.

Resisting temptation in a purchase fMRI task

Presenting Author: Henrik Walter

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Most established paradigms investigating neural responses to rewards are forced-choice tasks and depend heavily on attention and motor performance. We aimed to develop an innovative experimental task to represent the processes of rewarding decision-making where decisions for or against a monetary offer had to be made with little time constraints to examine the resistance of temptations.
In this fMRI task, 32 healthy male subjects (age: 24±3 years) underwent nine experimental courses with 20 trials each. Each trial had a different amount of money ranging from 1 to 99 cents but only five of the 20 offers could be "purchased" during each course. Subjects did not know which trials, higher or lower offers, were coming next. Subjects were paid the money they purchased at the end of the study. Regions of interest were those involved in self-control/inhibition with a focus on the lateral prefrontal cortex.
Resistance of temptation was operationalized as the interaction of value (high/low) and decision (purchase/decline). This contrast yielded significant clusters ($p<0.001$ corrected for multiple comparisons) in the dorsolateral prefrontal cortex bilaterally, the left anterior insula and the left anterior cingulate.
Here an innovative paradigm showed activity in networks involved in self-control/inhibition when resisting tempting monetary offers. We have implemented this paradigm in an ongoing NGFN study on withdrawal in alcohol addiction in order to investigate the neural correlates of self control in the withdrawal phase.

Identification of Low Prevalence Somatic Mutations in Heterogeneous Tumor Samples

Presenting Author: Edgar Funk (3)

Olivier Harismendy (1, 2), Lei Bao (1), Steve Kotsopoulos (3), Edgar Funk (3) Sophie Rozenzhak (1, 2), Jeff Olson (3), Masakazu Nakano (1, 2), Brian Crain (1), Stephanie Pond (4), Karen Messer (1), Richard Schwab (1), Mark S. Chee (4), Darren R. Link (3), Kelly A. Frazer (1, 2, 5).

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High throughput sequencing enables the digital measurement of each allele in a DNA sample. This provides an ideal method to interrogate mutations present in heterogeneous samples such as solid tumors in which clonal selection or contamination with stroma can hinder the identification of important somatic mutations. We developed an ultra-deep targeted sequencing (UDT-Seq) assay to screen 42 cancer genes via microdroplet-based PCR (RainDance Technologies) and direct sequencing of the amplicons on the Illumina GA. This UDT-Seq library interrogates ~86 kb of DNA located in cancer mutational hotspots (87% of all COSMIC database entries) and ~23 kb located in exons sequenced in HapMap samples for the assay calibration and performance evaluation.

We devised a statistical filtering of the mutations by using both experimental estimation of the sequencing error rate and training with a calibration sample. We measured the performance of our assay by processing 4 blends of 4 HapMap samples, interrogating 158 SNPs with known prevalence in each blend. The sensitivity and specificity of our method is >88% and >99% respectively for mutations present at 1% or greater. We next interrogated 4 cancer samples (xenografts, 2 of which with matching primary samples) from 4 different fresh frozen tissues. We were able to detect and validate low-prevalence somatic mutations in all samples of which some are well-known driver mutations. Finally, we analyzed the robustness of the detection and prevalence measurement after performing whole genome amplification and show that WGA leads to an underestimation of the mutant allele for mutations present at 5% prevalence or lower. Featuring a streamlined sample preparation to interrogate a large number of bases, this assay is well suited for clinical applications to study clonal selection in cancer progression or treatment with sub-optimal heterogeneous cancer samples.

Quantitative detection of circulating tumor DNA by droplet-based digital PCR.

Presenting Author: Edgar Funk (3)

Valerie Taly (2), Deniz Pekin (2), Steve Kotsopoulos (3), Edgar Funk (3), Xinyu Li (3), Ivan Atochin (3), Hu Gang (3), Delphine Le Corre (1), Leonor Benhaim (1), J. Brian Hutchison (3), Darren R. Link (3), Hélène Blons (1), Pierre Lurent-Puig (1).

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By segregating individual target DNA molecules into millions of aqueous droplets acting as independent microreactors, our procedure allows for extremely precise, sensitive, and fast quantification of mutated genes. The sensitivity of the procedure was confirmed by measuring 1/200,000 dilution of KRAS-mutated cell-line DNA in a background of wild-type DNA. Furthermore, plasma of more than 50 patients with metastatic CRC were tested. Our procedure enabled detection of the target mutation and the wild-type DNA, and thus, measurement of the total amplifiable DNA.

DNA concentration in the plasma samples varied by two orders of magnitude and was not correlated with the proportion of mutated DNA, which varied from 42% to 0.1%. By using a duplex analysis to detect either of the two most frequent mutations of KRAS (G12D and G13D) and the wild-type DNA, the expected mutation (known by primary tumor characterization) was detected in 16 out of the 19 samples. Two samples had a low amount of amplifiable DNA leading to an inconclusive result. Five samples, positive for the G13D mutation, were also tested for the G12D mutations and were negative. Moreover, we tested 54 plasma samples from patients with metastatic cancer with known KRAS status (mutated or not) in a multiplex format allowing the simultaneous analysis of the seven more frequent mutations of KRAS or the wild-type sequence. Among the mutated samples, 13 out the 19 mutated ones were positive and 32 of the non mutated ones were negatives.

Our results demonstrate that our digital PCR method enables non-invasive detection of KRAS in plasma of patients with metastatic CRC with high sensitivity and high specificity. We anticipate that the method will be employed in multiple applications in the clinic, including diagnosis, cancer recurrence monitoring, and treatment management.

Targeted Enrichment and Sequencing of Clinically Relevant Genes Using TargetRich™ Gene Panels

Presenting Author: Michael Ehret

Katie Jansen Spayd1 , Irina A. Vasenkova1 , Tatiana Shvetsova1 , David Kloske1 , Randy C. Bachmeyer1 , Richard M. Myers1,2 , D. Troy Moore1 , Katherine E. Varley1,2

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Next-generation sequencing (NGS) holds great promise for many clinical utilities, including personalized medicine, targeted therapy, fetal DNA screening, and the detection of mutations associated with rare genetic diseases. Most NGS applications target the whole genome or exome, resulting in wasted sequencer space and low coverage for clinically relevant regions of the genome. The transition of NGS into the clinic will require the ability to deeply sequence hundreds to thousands of targeted genetic regions from a large number of DNA samples.

Several teams have developed targeting (PCR) and capture (hybridization) methods for enrichment that are specific to a NGS platform. The drawbacks of many of these methods include the requirement of large quantities of patient DNA, reduced sequencing capacity due to wasted off-target sequencing, high cost of reagents, low sample throughput, the requirement of specialized equipment, and inflexible design rules. We developed TargetRich gene panels employing Nested PatchPCR™, for investigators to perform targeted sequencing of hundreds of genomic regions in large numbers of patient samples, including formalin fixed paraffin embedded samples (FFPE), to fully and efficiently utilize NGS platforms.

Here, we describe the use of two TargetRich cancer panels: CRX (for targeted enrichment of exons from 10 genes) and CR63 (for targeted enrichment of exons from 63 genes). In a single tube, from as little as 10 ng of patient DNA, we simultaneously amplified over 750 exons. The TargetRich Nested PatchPCR protocol can be completed in a single day, requires no specialized laboratory equipment, and is amenable to high-throughput sample preparation. We sequenced the targeted genes on three NGS platforms: Illumina GAIIx®, MiSeq® and Ion Torrent PGM®. The high percentage of on-target sequence data and focused content allowed us to achieve high coverage across the loci (average of 400X read depth). The accuracy of variant detection was confirmed using deeply sequenced genomic DNA from the 1000 Genomes Project. In addition, we detected variants at frequencies of <1% in a mixed population, demonstrating the high sensitivity of the protocol. Together, these results demonstrate the potential utility for Nested PatchPCR for targeted enrichment in a variety of clinical applications.

National Genome Research Network

Company Satellite Sessions

Abstracts

From basic research to clinical samples - When are microarrays the right tool for the job?

Presenting Author: Maik Pruess

Affymetrix Europe

The predicted death of microarray technology has still not happened because Next Generation Sequencing (NGS), like every other technology before it, does some things very well and others less well.
However, the way in which microarrays are best applied has evolved and the smartest science is now achieved by combining the strengths of NGS, microarrays and other technologies to meet research objectives in a timely, robust and cost-efficient way.
We will discuss basic research, and translational research in clinical sample types to provide an update on what we are doing to ensure that, when a microarray or other complementary technology is the best tool for the job, you should speak to us at Affymetrix to enable your research.

Automation of NGS Sample Preparation: From Benchtop NGS to Genome Centers

Presenting Author: Hannes Arnold, PhD, Business Development Manager NGS Europe

Caliper - a PerkinElmer Company

With the advent of Next Generation Sequencing (NGS) systems, the landscape of sequencing applications in molecular biology has completely changed. The tremendous increase of sequencing capacities of NGS devices in combination with enrichment strategies for targeted resequencing creates a bottle-neck in library preparation. Sample preparation is the key to high quality data for nucleic acid sequencing. NGS sample preparation protocols can be complex and involve repetitive process steps which are each simple, but need to be executed carefully to generate high yields of consistent quality libraries. Automation solutions based on liquid handling platforms and micro fluidic systems do not only allow increased throughput for sample preparation but also enable consistently high quality, performance and robustness in library preparation. With NGS applications expanding from research into translational medicine and clinical settings, the major focus in automation of NGS sample preparation is moving beyond increased throughput toward better process control, and improved ease of use. In this presentation, we will provide an overview of PerkinElmer's solutions for automated NGS sample preparation for high end sequencing systems typically used in research settings as well as those for small bench top systems.

Illumina's Genomics Research Portfolio: From Whole Genomes to Targeted approaches

Presenting Author: Luc Smink, Regional Marketing Manager, Sequencing & Informatics, EMEA,

Illumina UK Ltd

The first human genome sequence was the goal of the Human Genome Project, as Renato Dulbecco stated his wish: "To sequence the human cellular genome with a view of understanding the cancer genome". Today that first human genome in conjunction with NGS sequencing and high-throughput Genotyping arrays has changed the face of Genomics. The arrays and NGS have contributed hugely to our understanding of Human Disease.

Illumina's NGS sequencing technology as well as the Illumina's Genotyping array's have played and continue to play a key part in a number of large-scale genome projects such as the International Cancer Genome Consortium, 1000 Genomes Project and the Encode Project. The innovation in our sequencing platforms has driven higher throughput, longer read lengths, easier workflows, faster run times, as well as integrated software solutions, all at very high accuracy and across our portfolio. From the high throughput HiSeq2500/1500, to the lower throughput MiSeq, providing the ability to sequence and detect new variants and mutations. Discoveries, that also contribute new content to our genotyping arrays.

Please come and join us to learn about the latest news across our portfolio as well as the broad range of applications they enable, from small scale targeted approaches with dedicated panels, to Genome-wide approaches, as well as tools to study the Transcriptome and its regulation.

Droplet Digital PCR: Molecular Biology in High Resolution

Presenting Author: Pia Scheu, ddPCR Sales Specialist Europe

Bio-Rad Laboratories GmbH

Droplet digital™ PCR (ddPCR™) is the third generation of PCR technology. The QX100 ddPCR system from Bio-Rad Laboratories provides an absolute measure of target DNA molecules with unrivaled accuracy, precision, and sensitivity.
In the QX100 ddPCR system, the target molecules in a DNA sample are partitioned into 20000 nanodroplets. The goal is to have either one or no molecules in each droplet, so that when we perform PCR we have a simple positive or negative amplification result. The initial amount of target molecules is then directly quantified by counting the number of droplets with a positive amplification.
Droplet digital PCR provides researchers with a new tool for the precise measurement of copy number variation, low-abundance sequence detection, detection of rare mutations, including distinguishing rare sequences in tumors, and gene expression analysis.

Ion torrent next Generation sequencing technology: the revolution of the revolution

Presenting Author: Raimo Tanzi, Instrument District Leader - Next Gen Sequencing – Europe

Life Technologies GmbH

Next Generation Sequencing technology has been unanimously recognized as a revolutionary technology which enabled, in the last 5 years, an incredible wave of progress in science and publications. Ion torrent is a revolutionary way to do next Generation Sequencing, characterized by a number of features and benefits which do not belong to classical, light based, detection methods.
The fundamental principle of Ion torrent detection method is to use the signal of protons generated by the reaction, differently from all other molecular biology techniques which use photons. This was made possible by the introduction, for the first time in history, of semiconductors technology into molecular biology, as a substitute for traditional optical detection systems. Among the practical benefits associated to this innovation we can list the simplification of the whole process, the scalability of the microchips based system, the speed of real time detection, the great reduction of costs.
Ion torrent technology is today still in its second year of existence and has already proven to be effective in multiple applications, from microbiology to transcriptomics, from targeted resequencing of gene panels to de Novo sequencing of whole genomes. With the introduction of 100 fold higher production level chips on the Proton system, the number of available applications will grow to include ultra high throughput sequencing of whole exomes, whole transcriptomes, Chip-Seq and , finally , whole genomes.

Whole exome sequencing (WES) speeds up gene identification in monogenetic human diseases

Presenting Author: Janine Altmüller

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Advanced sequencing technologies have influenced genomic research dramatically in the last 5 years. Whereas sequencing of the first human genome needed tremendous efforts, time, and expense, re-sequencing of an individual's exome (WES) can nowadays be done in a very short time period and for less than € 1,000.
WES focuses on about 1% of the human genome which is believed to be the functionally most relevant part - leading to the identification of causative variants for monogenetic diseases, relevant pathways for complex diseases and driver mutations in cancer genomes.
This presentation will address project design aspects of different applications, technical issues and technology comparison, our analysis pipeline including variant filtering and prioritization as well as recent results of selected whole exome sequencing projects.

Translating Genomic Discovery into Human Health - The MassARRAY® for Somatic Mutation Profiling in Cancer and Quality Control for NGS Projects

Presenting Author: Rebekka Krumbach and Susanne Mueller

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Patient-derived xenografts (PDX) established in serial passage in nude or SCID mice closely mimic patient tumor properties, making them excellent models for therapeutic and biological studies. With the advent of patient tailoring and targeted therapies, detailed knowledge of the molecular make-up of the tumor models is essential.
Oncotest has analyzed the complete panel of > 300 PDXs for mutations (OncoCartaTM panels and Sanger sequencing), gene expression and copy number variation. Knowledge of these molecular characteristics is used to move forward from comparing response to therapy between histologies, to comparing response in tumors differing in a molecular marker. This will be demonstrated on the example of BRAF and MEK inhibitor anti-tumor activity in melanoma differing in their mutational profiles. This example shows how knowledge of mutations in a panel of tumor models can be used to answer questions about sensitivity and resistance to cancer therapeutics.
In addition to the 3 generic OncoCartaTM panels, the new LungCartaTM panel allows the analysis of key somatic mutations identified via sequencing discovery studies that affect key pathways in lung adenocarcinoma tumors. It offers a comprehensive screen of 26 tumor suppressors and oncogenes and detects 214 mutations in EGFR, KRAS, HER2, MET, and more. The small-sized amplicons (typically <120 bp) make it compatible with degraded FFPE samples. Only ~240 ng DNA per sample are required and mutations with as low as 5% frequency are detected and quantified.
Correct sample tracking is a vital factor in comparison of normal vs tumor cells as well as in genome-wide association studies and next gen sequencing experiments. The iPLEX® SampleID panel is a single well test containing 50 assays. Parallel genotyping of 42 SNPs enables a high degree of discriminatory power of > 1.00 x 10-18, while 3 assays target chromosome X/Y paralogs for sex determination and 5 assays assess the number of amplifiable template DNA copies via Real-Competitive PCR. Dedicated software allows plate-to-plate comparison, historical lookup with all samples in a database and tumor versus normal comparison. It identifies chain-ofcustody events, expected matches as well as unexpected matches. Results are available in both table and html format.

Gene Expression Analysis Down to the single Cell level by Digital Quantification of Nucleic Acids Utilizing a Color-Coded Barcode Technology

Presenting Author: Jim White, PhD, Field Application Scientist

Nanostring Technologies Europe

The nCounter System from NanoString Technologies, uses direct single molecule imaging with molecular barcodes to detect up to 800 targets in a single reaction direct from total RNA. The assay technology captures nucleic acid targets through hybridization, eliminating the need for reverse transcription or amplificaton by PCR.
The multiplex probe assay or CodeSet, reduces pipetting and the need for multiple reactions. The input is 100ng total RNA, there is no loss of sample from aliquoting, no gaps in data from gene dropouts, five¬-log dynamic range, reproducible less than integer fold change. Publications show high correlations between sample types, including FFPE samples or data from other platforms such a NGS and peer-to-peer studies.
The introduction of the nCounter® Single Cell Gene Expression Assay, allows researchers to study entire biological pathways for single cells without the necessity to match the gene number to the format of a microfluidic PCR consumable. In contrast to the standard protocol a linear amplification step will be performed prior to the final detection. The Multiplexed Target Enrichment (MTE) step uses primers that flank the region targeted by the nCounter Capture and Reporter probes, and allows transcripts within individual cells to be linearly amplified after a reverse transcription step. MTE can linearly amplify up to 800 targets from a single cell in a single tube without bias.
The resulting amplified material is then directly hybridized with an nCounter CodeSet targeting the genes of interest - no sample clean-up or sample splitting is required.
The system supports applications for copy number Analysis, ChIP-String-epigenetic analysis, miRNA profiling and gene expression analysis down to the single cell level.

National Genome Research Network

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Wieben	Eric	Prof. Dr.	Mayo Clinic, Rochester, MN	wieben@mayo.edu
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Winands	Armin	Dir. Sales& Support	Ingenuity Systems	awinands@ingenuity.com
Winkler	Thomas		Institute of Epidemiology and Preventive Medicine, Regensburg	thomas.winkler@klinik.uni-regensburg.de
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Zeller	Tanja	Dr	University Heart Center Hamburg	t.zeller@uke.de
Zimmer	Ralf	Prof. Dr.	LMU, München	zimmer@ifi.lmu.de
Zimmer	Andreas	Prof. Dr.	University of Bonn	neuro@uni-bonn.de
Zimmermann	Martina	Dr. oec. troph	Universitätsklinikum Regensburg	martina.zimmermann@ukr.de
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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.	Deutsches Herzzentrum München	E1, F1	**E1** SNP array for atherosclerosis Development of innovative diagnostics **F1** Coordinating office
Erdmann	Jeanette	Prof. Dr. rer. nat.	Universität zu Lübeck	A1a	**A1** Polygenic and monogenic forms of MI
Hengstenberg	Christian	Prof. Dr. med.	Universität zu Regensburg	A1b, C1	**A1** Polygenic and monogenic forms of MI **C1** Cases and population platform (KORA/MONICA; GMIS;PREVENT-IT, LE HEART)
Diemert	Patrick	PD Dr. med.	Universität zu Lübeck	A2a, D1, D2a	**A2** Genomics of coronary artery disease **D1** Gene expression profiling Transcriptome of monocytes in subclinical atherosclerosis and MI patients **D2** Genomics of plasma lipids
Fischer	Marcus	PD. Dr. med.	Universität zu Regensburg	A2b, D2b	**A2** Genomics of coronary artery disease **D2** Genomics of plasma lipids
Blankenberg	Stefan	Prof. Dr. med.	Universitätsklinikum Hamburg Eppendorf	A3a, D1	**A3** Genomics of sub clinical atherosclerosis **D1** Gene expression profiling Transcriptome of monocytes in subclinical atherosclerosis and MI patients
Koenig	Wolfgang	Prof. Dr. med.	Universitätsklinikum Ulm	A3b, E2	**A3** Genomics of sub clinical atherosclerosis **E2** 50 K Vascular Disease SNP Array
Teupser	Daniel	Prof. Dr. med.	Klinikum der Universität München	B1	**B1** Syntenic regions for atherosclerosis in mice and humans
Aherrarhou	Zouhair	Dr. med.	Universität zu Lübeck	B2	**B2** ABCC6 and arterial calcification
Steller	Ulf	Dr. rer. nat.	Euroimmun AG	E1	**E1** SNP array for atherosclerosis Development of innovative diagnostics
Ziegler	Andreas	Prof. Dr. rer. nat.	Universität zu Lübeck	C2b	**C2** Genetic epidemiology methods platform
Meitinger	Thomas	Prof. Dr. med.	Helmholtz Zentrum München	CF	**CF** Genotyping/sequencing facility
Hübner	Norbert	Prof. Dr.	Max-Delbrück-Centrum für molekulare Medizin		
IG Genetics of Heart Failure (Genetik der Herzinsuffizienz) Koordination: Prof. Dr. Hugo A. Katus					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	1a	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.	Charité 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	PD Dr.	Universitätsklinikum Heidelberg	4*	Genetic Modifiers of Heart Failure in Mice
Rottbauer	Wolfgang	Prof. Dr.	Universitätsklinikum Ulm	5	Functional Genomics in Zebrafish to Dissect the Genetics of Human Myocardial Disease
Frey	Norbert	Prof. Dr.	Universitätsklinikum Heidelberg	6	Novel Molecular Pathways in Cardiac Hypertrophy and 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	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 Arteriosklerosеforschung an der Universität Münster	10	Genetic epidemiology of Heart Failure: Genetic Epidemio-logical Support for the IG
Eils	Roland	Prof. Dr.	Deutsches Krebsforschungszentrum	11	Bioinformatic Methods
Meder	Benjamin	Dr.	Universitätsklinikum Heidelberg	12	DNA-Platform
Rottbauer	Wolfgang	Prof. Dr.	Universitätsklinikum Ulm	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.**	**Titel**
Hinney	Anke	Prof. Dr.	Universität Duisburg-Essen	TP1	Identification of human obesity genes with a focus on developmental aspects
Schürmann	Annette	Prof. Dr.	Deutsches Institut für Ernährungsforschung (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	Prof. 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	Prof. 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 Berlin	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	Heiner	Prof. Dr.	Deutsches Institut für Ernährungsforschung (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	PD Dr.	Charité Berlin	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.**	**Titel**
Haas	Jürgen G.	Prof. Dr. Dr.	LMU München	1	Herpesviral factors modulating the cellular miRNA processing machinery
Koszinowski	Ulrich	Prof. Dr.	LMU München	2	Characterization of CMV miRNAs in vitro and in vivo
Dölken	Lars	Dr.	LMU München	2	Characterization of CMV miRNAs in vitro and in vivo
Adler	Heiko	Prof. Dr.	Helmholtz-Zentrum München	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	Prof. Dr.	Universität Regensburg	5	Identification of cellular targets of viral miRNAs
Förstemann	Klaus	Prof. Dr.	LMU München	6	Biochemical interaction of viral and cellular miRNAs
Zimmer	Ralf	Prof. Dr.	LMU München	7	Prediction of viral miRNAs targets

IG RNomics in Infections

Koordination: Prof. Dr. Jürgen Brosius

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Reinhardt	Richard	Dr.	MPI für Molekulare. Genetik	1	Ultra-High-Parallel Sequencing and Biocomputational Analysis of npcRNA
Vogel	Jörg	Prof. Dr.	Julius-Maximilians-Universität Würzburg	2a	RNomics of bacterial infections
Rudel	Thomas	Prof. Dr.	Universität Würzburg	2b	RNomics of bacterial infections
Walter	Lutz	Prof. 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.**	**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
Weidinger	Stefan	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
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
Rosenstiel	Philip	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 9	Funktionelle Aufklärung
Rosenstiel	Philip	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 10	Systematische Aufklärung von Signaltransduktions-wegen: angeborene Immunität
Franke	Andre	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T1	Qualitätsmanagement
Wittig	Michael		Uniklinik Schleswig-Holstein, Campus Kiel	T3a	Bioinformatische Unterstützung
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
Kabesch	Michael	Prof. Dr.	Medizinische Hochschule Hannover	GP 2	Genetische Ätiologie des Asthma bronchiale
Horstmann	Rolf	Prof. Dr.	Bernhard-Nocht-Institut für Tropenmedizin	GP 4	Genetischer Ätiologie der Tuberkulose
Lee	Young-Ae	Prof. Dr.	Charité, Campus Virchow-Klinikum	GP 5	Genetische Ätiologie der atopischen Dermatitis
Manke	Thomas	Dr.	Max-Planck-Institut für Immunbiologie, Freiburg	T 3b	Bioinformatische Unterstützung
Albrecht	Mario	Dr.	Max Planck Institut für Informatik (MPI-INF)	T 3c	Bioinformatische Unterstützung
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	GP11	Systematische Aufklärung von Stoffwechselwegen - Adaptive Immunität;
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	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.**	**Titel**
Döhner	Hartmut	Prof. Dr.	Uni Ulm	TP1	Identifizierung und Charakterisierung pathogenetisch relevanter Gene bei der akuten myeloischen Leukämie (AML)
Thiede	Christian	Prof. Dr.	TU Dresden	TP2	Identifikation neuer molekularer Veränderungen bei Patienten mit akuter myeloischer Leukämie und normalem Karyotyp
Hubert	Serve	Prof. Dr.	Uni Frankfurt	TP3a	Epigenetics of AML
Müller-Tidow	Carsten	Prof. Dr.	Uni Münster	TP3b	Epigenetics of AML
Kulozik	Andreas	Prof. Dr.	Uni Heidelberg	TP4	NOTCH1 signaling
Marschalek	Rolf	Prof. Dr.	Uni Frankfurt	TP5	MLL and stem cell program
Bohlander	Stefan	Prof. Dr.	LMU München	TP6	CALM/AF10 target gene analysis
Feuring-Buske	Michaela	PD. Dr.	Universität Ulm	TP7	MEIS1 homeobox gene expression

Leutz	Achim	Prof. Dr.	MDC Berlin	TP8	Wnt signaling in leukemic stem cells
Duyster	Justus	Prof. Dr.	TU München	TP9	Genetic basis of imatinib resistance
Grez	Manuel	Prof. Dr.	GSH Frankfurt	TP10	Molecular inhibitors of AML1/ETO
Neubauer	Andreas	Prof. Dr.	Uni Marburg	TP11	Resistance to retinoic acid in AML
Schrappe	Martin	Prof. Dr.	Uni Kiel	TP12	Very high risk childhood ALL
Lottaz	Claudio	Dr.	Uni Regensburg	TP15	Bioinformatics, clinical data, and leukemic cell banks
Karawajew	Leonid	Dr.	Charité Berlin	TP13	Molecular dissection, functional evaluation and preclinical targeting of intermediate-risk childhood acute lymphoblastic leukemia
Hagemeier	Christian	Prof. Dr.	Charité Berlin	TP14 TP16	Systematic approaches towards genes with pathogenetic, prognostic and therapeutic value in relapsed acute lymphoblastic leukemia in children

IG Brain Tumor Network

Koordination: Prof. Dr. Peter Lichter

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Lichter	Peter	Prof. Dr.	DKFZ Heidelberg	SP-C	Koordinierung und Lenkung des Netzwerkes BTN^{plus}
Lichter	Peter	Prof. Dr.	DKFZ Heidelberg	SP-1	Hochdurchsatzanalyse von potentiellen Onkogenen und Tumorsuppressorgenen in Gliomen
Wolter	Marietta	Dr.	Heinrich-Heine-Universität, Düsseldorf	SP-2a	Aberrante miRNA-Expression in Gliomen: Molekulare Mechanismen, funktionelle Konsequenzen und deren klinische Signifikanz
Stühler	Kai	Prof. Dr.	Ruhr-Universität Bochum	SP-2b	Aberrante miRNA-Expression in Gliomen: Molekulare Mechanismen, funktionelle Konsequenzen und deren klinische Signifikanz
Brors	Benedikt	Dr.	DKFZ Heidelberg	SP-3	Modellierung und Bioinformatik
Lichter	Peter	Prof. Dr.	DKFZ Heidelberg	SP-4	Funktionelle Charakterisierung der an Hypoxie und Sauerstoffmetabolismus 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 Heidelberg	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 Krebsstammzellen in hochgradigen Gliomen
Beier	Christoph	Dr.	Technische Hochschmmule Aachen	SP-7b	Gestörte Migration und Differenzierung – die Rolle von ZEB1 im Rahmen der Malignisierung von neuralen Stammzellen in Tumorstammzellen des Glioblastoms
Waha	Andreas	PD Dr.	Universitätsklinikum Bonn	SP-8	Funktionelle Bedeutung epigenetisch deregulierter Gene in Gliomen
Angel	Peter	Prof. Dr.	DKFZ Heidelberg	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	PD Dr.	DKFZ Heidelberg	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 Zellteilungs-symmetrie in malignen Gliomen kontrollieren
Radlwimmer	Bernhard	Dr.	DKFZ Heidelberg	SP-11b	Molekulare und funktionelle Charakterisierung von Genen, welche die Zellteilungs-symmetrie in malignen Gliomen kontrollieren
Herold-Mende	Christel	Prof. Dr.	Universität Heidelberg	SP-12a	Funktionelle Analysen von differenzierungsrelevanten Kandidatengenen in Gliomstammzellen
Radlwimmer	Bernhard	Dr.	DKFZ Heidelberg	SP-12b	Funktionelle Analysen von differenzierungsrelevanten Kandidatengenen in Gliom-Stammzellen
Hartmann	Christian	PD Dr.	DKFZ Heidelberg	SP-13	Funktionelle Charakterisierung der putativen Tumor-suppressorgene *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-Auto-antigen-Kandidat mit immuno-suppressiven Eigenschaften

IG Integrated Genome Network of Prostate Cancer Koordination: PD Dr. Holger Sültmann					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Schlomm	Thorsten	Prof. Dr. med.	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 Mikroamplifikationen im Prostatakarzinom
Dierlamm	Judith	Prof. Dr. Dr.	UKE Hamburg-Eppendorf	TP3*	Zytogenetische und molekulare Charakterisierung von Translokations-Bruchpunkten im Prostatakarzinom
Schweiger	Michal-Ruth	Dr. Dr.	MPI für Molekulare Genetik	TP4*	Analyse von Mutationen und epigenetischen Veränderungen im Prostatakarzinom
Sültmann	Holger	Prof. Dr.	Deutsches Krebsforschungszentrum	TP5*	Splice-Varianten- und miRNA Expression in Tumoren
Kuner	Ruprecht	Dr.	Deutsches Krebsforschungszentrum	TP5*	Splice-Varianten- und miRNA Expression in Tumoren
Balabanov	Stefan	Dr. med. Dr.rer nat.	Universitätsklinikum Hamburg Eppendorf	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 Krebsforschungszentrum	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
Sültmann	Holger	Prof. Dr.	Deutsches Krebsforschungszentrum	TP11*	Funktionelle zelluläre Assays in Prostatakarzinom-Zelllinien
Sültmann	Holger	Prof. Dr.	Deutsches Krebsforschungszentrum	TP12*	In vivo Analyse von Genen im Prostatakarzinom
Beissbarth	Tim	Prof. Dr.	Deutsches Krebsforschungszentrum	TP13*	Bioinformatik und Systembiologie
Sültmann	Holger	Prof. Dr.	Deutsches Krebsforschungszentrum	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.**	**Titel**
Berthold	Frank	Prof. Dr.	Klinikum der Universität zu Köln, Zentrum für Kinderheilkunde 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 Krebsforschungszentrum	3	Identification of NB initiating cells
				14	Targeting class I histone deacetylases
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	1	Project management
				4a	Proteomics of NB master regulators
				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	PD Dr.	Institut für Pharmazie und Molekulare Biotechnologie/Bioquant	12	Refined treatment selection with machine learning techniques
Lawerenz	Christian	Dr.	Deutsches Krebsforschungszentrum	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 Krebsforschungszentrum	10	NB Fragilome
Schramm	Alexander	PD 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	Prof. Dr.	Ruhr-Universität Bochum	4b	Proteomics of NB master regulators
Westermann	Frank	Dr.	Deutsches Krebsforschungszentrum	13	Targeting Myc functions
Witt	Olaf	Prof. Dr.	Deutsches Krebsforschungszentrum	3	Identification of NB initiating cells
				14	Targeting class I histone deacetylases

IG Deciphering Oncogene Dependencies in Human Cancer Oncogene Mutation Space Koordination: Prof. Dr. Roman Thomas					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Thomas	Roman	Prof. 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	4	Functional impact of oncogene mutants and small molecules on the Ras and Rho signaling pathways
Wittinghofer	Alfred	Prof. Dr.	MPI für molekulare Physiologie	4	Functional impact of oncogene mutants and small molecules on the Ras and Rho signaling pathways
Rauh	Daniel	Prof. Dr.	MPI for Molecular Physiology Dortmund	5	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.**	**Titel**
Lange	Bodo	PD Dr.	Max-Planck-Institut für Molekulare Genetik	TP1*	Project coordination
Lehrach	Hans	Prof. Dr.	Max-Planck-Institut für Molekulare Genetik	TP1*	Project coordination
Brand	Angela	Prof. Dr.	Maastricht University	TP2*	Translational Health Research
Schweiger	Michal-Ruth	Dr. Dr.	Max-Planck-Institut für Molekulare Genetik	TP3*	Mutational analysis
Mollenhauer	Jan	Prof. Dr.	Medical Biotechnology Center University of Southern Denmark	TP4*	Recombinant cancer cell libraries & drug target recovery
Sültmann	Holger	Prof. Dr.	German Cancer Research Center (DKFZ)	TP5*	Quantification of cancer pathways
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	TP6*	Protein interaction networks
Schäfer	Reinhold	Prof. Dr.	Charité Universitätsmedizin Berlin	TP7*	Cellular signalling networks
Herrmann	Bernhard	Prof. 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	Dr.	Max-Planck-Institut für Molekulare Genetik	TP10*	Data integration and modelling
Drewes	Gerard	PD 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.**	**Titel**
Gress	Thomas M.	Prof. Dr.	Philipps-Universität Marburg	TP0,TP1b, TP2b,	TP0 Koordination TP1b Klinische Ressourcen und Daten TP2b Mausmodelle des Pankreaskarzinoms
Giese	Nathalia	Dr.	Universitätsklinikum Heidelberg	TP1a	Klinische Ressourcen und Daten
Tannapfel	Andrea	Prof. Dr.	Ruhr-Universität Bochum	TP1c	Klinische Ressourcen und Daten
Sipos	Bence	Prof. Dr.	Eberhard-Karls Universität Tübingen	TP1d	Klinische Ressourcen und Daten
				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
Hoheisel	Jörg	Dr.	DKFZ Heidelberg	TP5, TP12	TP5 Quantitative Analyse von Proteininteraktionen TP12 Epigenetische Analyse zur therapeutischen Patienten-Stratifizierung
Hahn	Stephan	Prof. Dr.	Ruhr-Universität Bochum	TP6	MiRNAs als therapeutische Targets für das Pankreaskarzinom
Kleeff	Jörg	Prof. Dr.	TU München	TP7	Molekulare Analyse der tumorspezifischen Stromaaktivierung
Schwarte-Waldhoff	Irmgard	PD Dr.	Ruhr-Universität Bochum	TP9a	Entwicklung von molekulardiagnostischen Verfahren zur Früherkennung des Pankreaskarzinoms basierend auf sezernierten/freigesetzten Kandidaten-Proteinen
Schnölzer	Martina	Dr.	DKFZ Heidelberg	TP9b*	Entwicklung von molekulardiagnostischen Verfahren zur Früherkennung des Pankreaskarzinoms basierend auf sezernierten/freigesetzten Kandidaten-Proteinen
Kestler	Hans	Prof. Dr.	Uniklinik Ulm	TP11b	Molekulare Differentialdiagnose
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.**	**Titel**
Schweiger	Michal-Ruth	Dr. 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 epigenetischen 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-Sequenzierungsplatform 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.**	**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*	Systems biology of signaling pathways in colorectal carcinomas
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.	Deutsches Krebsforschungszentrum	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.**	**Titel**
Cichon	Sven	Prof. Dr. rer. nat.	Universitätsklinikum Bonn	1	Genomik bei Bipolarer Störung
Holsboer	Florian	Prof. Dr. Dr.	Max Planck Institut für Psychiatrie	2	Genomik bei unipolarer Störung
Rujescu	Dan	Prof. Dr.	Psychiatrische Klinik der LMU	3	Genomik bei Schizophrenie
Maier	Wolfgang	Prof. Dr.	Universitätsklinikum Bonn	3b	Genomik bei Schizophrenie
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 Seelische Gesundheit	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Meyer-Lindenberg	Andreas	Prof. Dr.	Zentralinstitut für Seelische Gesundheit	6a	Imaging Genetik
Walter	Henrik	Prof. Dr. med. Dr. phil.	Universitätsmedizin Charite, Campus Mitte	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	Prof. Dr. rer. nat.	Universitätsklinikum Bonn	9	Allel-spezifische Expression
Eils	Roland	Prof. Dr.	DKFZ 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 Mausmodellen und Proteomanalysen
Wurst	Wolfgang	Prof. Dr. rer. nat.	Helmholtz Zentrum München	12b	Funktionelle Untersuchungen mit Hilfe von transgenen Mausmodellen und Proteomanalysen
Deussing	Jan	Dr.	Max Planck Institute of Psychiatry	12b	Funktionelle Untersuchungen mit Hilfe von transgenen Mausmodellen 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

IG Genetics of Alcohol Addiction

Koordination: Prof. Dr. Rainer Spanagel

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Spanagel	Rainer	Prof. Dr.	Central Institute of Mental Health	1	Coordination Consortium
Eils	Roland	Prof. Dr.	German Cancer Research Center	2a	Gene data mining platform and statistics
Wienker	Thomas	Prof. Dr.	University of Bonn	2b	Gene data mining platform and statistics
Matthäus	Franziska	Dr.	University of Heidelberg,	3	Mathematical Modelling and Analysis
Schütz	Günter	Prof. Dr. med.	German Cancer Research Center (DKFZ)	4	Functional analysis I and conditional mouse models
Wurst	Wolfgang	Prof. Dr.	GSF - National Research Center for Environment and Health	5	Functional analysis II and RNAi in vivo application
Zimmer	Andreas	Prof. Dr.	University of Bonn	6	Functional analysis III
Bartsch	Dusan	Prof. Dr.	Central Institute of Mental Health	7	Transgenic rat models
Zimmer	Andreas	Prof. Dr.	University of Bonn	8	Behavioral analysis of Animal Models
Spanagel	Rainer	Prof. Dr.	Central Institute of Mental Health	8	Behavioral analysis of Animal Models
Gebicke-Haerter	Peter	Prof. Dr.	Central Institute of Mental Health	9	Glutamatergic and epigenetic profiling with microarrays
Sprengel	Rolf	Dr.	MPI Med. Forschung Heidelberg	10	Transcriptional and posttranscriptional modifications
Rietschel	Marcella	Prof. Dr.	Central Institute of Mental Health	11	GWA studies in alcohol dependent patients and replication studies
Nöthen	Markus	Prof. Dr.	University of Bonn	11	GWA studies in alcohol dependent patients and replication studies
Dahmen	Norbert	PD Dr.	Universität Mainz	12a	GWA studies in population-based samples for high versus low alcohol consumption and replication studies

Wichmann	H. Erich	Prof. Dr.	GSF Institute of Epidemiology	12b	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	13b	Endophenotyping with fMRI: Genetic modulation and treatment response
Walter	Henrik	Prof. Dr.	Universitaetsmedizin Charite, Campus Mitte	13a	Endophenotyping with fMRI: Genetic modulation and treatment response
Kiefer	Falk	Prof. Dr.	Central Institute of Mental Health	13c	Endophenotyping with fMRI: Genetic modulation and treatment response
Mann	Karl	Prof. Dr. Dr.	Central Institute of Mental Health	14a	Endophenotyping with spectroscopy: Genetic modulation and treatment response
Gallinat	Jürgen	Prof. Dr.	Psychiatry, Charité, CCM	14b	Endophenotyping with spectroscopy: Genetic modulation and treatment response
Sartorius	Alexander	Prof. apl.	Central Institute of Mental Health	15*	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.**	**Titel**
Reis	André	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, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	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	Prof. 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.**	**Titel**
Kubisch	Christian	Prof. Dr.	University of Ulm	1	Genome-wide association analysis and gene identification in migraine with aura
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. med. habil.	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	Prof. Dr.	University of Bonn	5	Genetic basis of Levetiracetam pharmacoresistance and side effects in human epilepsy (TP5)
Lerche	Holger	Prof. Dr.	Universitätsklinikum Tübingen	6	Functional analysis of human ion channel mutations in cellular and animal models (TP6)
Becker	Albert	Prof. 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	Prof. Dr.	University of Hamburg	9	Subthreshold ion channels in epileptogenesis and neuronal synchronization

IG Gene Identification and Functional analyses in Alzheimer's disease

Koordination: Prof. Dr. Matthias Riemenschneider

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Riemenschneider	Matthias	Prof. Dr	Universitätsklinikum des Saarlandes	1	Identification of genetic factors in Alzheimer's disease
Bertram	Lars	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	3	The physiological function of BACE1-is BACE1 a safe therapeutic target
Garratt	Alistair	Dr.	Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch	3	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 Universität 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 (Dr. Thomas Floss)	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	9	Animal models for candidate genes of Alzheimer's disease
Riemenschneider	Matthias	Prof. 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.**	**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	Prof. Dr.	DZNE München	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	Modifier screen in flies overexpressing LRRK2
Zweckstetter	Markus	Prof. Dr.	Max-Planck-Institut für Biophysikalische Chemie	TP6	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

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
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
Ueffing	Marius	Prof. 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	TP13	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 (Dr. Daniela Vogt-Weisenhorn)	Wolfgang	Prof. Dr.	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.**	**Titel**
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	1*	Protein-Protein Interaktionsnetzwerke bei neurodegenerativen Erkrankungen
Selbach	Matthias	Prof. 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 Protein-komplexkomposition und Funktion durch Stress und Neurodegenerative Krankheitssignale
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	6a*	Erstellung von Genexpressionssignaturen von neurodegenerativen Krankheitsprozessen
Nietfeld	Wilfried	Dr.	Max-Planck-Institut für Molekulare Genetik	6b*	Erstellung von Genexpressionssignaturen von neurodegenerativen Krankheitsprozessen
Bork	Peer	Prof. Dr.	European Molecular Biology Laboratory	6c*	Erstellung von Genexpressionssignaturen von neurodegenerativen Krankheitsprozessen
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.**	**Titel**
Stewart	Francis	Prof. Dr.	Technische Universität Dresden	1	Genidentifikation und DNA Konstruktproduktion
von Melchner	Harald	Prof. Dr.	Universität Frankfurt	2	In situ Markierung von Krankheitsproteinen in embryonalen Stammzellen mit Genfallen-induzierten Mehrzweckallelen
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	3	Produktion proteinmarkierter pluripotenter und differenzierter ES Zellen
Hyman	Tony	Prof. Dr.	MPI für Zellbiologie und Genetik Dresden	4	Produktion und Imaging von HeLa und ES Zelllinien
Brüstle	Oliver	Prof. Dr.	Universität Bonn	5	Etablierung und Analyse transgener hES Zelllinien und neuralen Stammzelllinien
Mann	Matthias	Prof. Dr.	MPI für Biochemie, Martinsried	6	Proteininteraktionsstudien mittels massenspektrometrie-basierter Proteomik in in vitro und in vivo Systemen
Hansen	Jens	Dr.	Helmholtz Zentrum München	7a	DiGtoP bioinformatics – resource development and application in comparative network analysis

Gibson	Toby	Prof. Dr.	EMBL Heidelberg	7	DiGtoP bioinformatics – resource development and application in comparative network analysis
Kühn	Ralf	Dr.	Helmholtz Zentrum München	8	Mausmodelle für die in vivo Validierung von Proteininteraktionen
Buchholz	Frank	Prof. Dr.	MPI für Zellbiologie und Genetik Dresden	9	Validierung und Zergliederung der Signalwege von Krankheitsrelevanten Genen mit endoribonucelase präparierter siRNA
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	10	Management & Training

IG German Mouse Clinic (GMC)

Koordination: Prof. Dr. Martin Hrabě de Angelis

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	1	Core Facility
Wolf	Eckhard	Prof. Dr.	Genzentrum der LMU München	2	Clinical Chemical Screen
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	3	Behavioral Screen
Klopstock	Thomas	PD Dr. med.	LMU München	4	Neurological Screen
Graw	Jochen	Prof. Dr.	Helmholtz Zentrum München	5	Eye Screen
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	6	Dysmorphology Screen
Busch	Dirk	Prof. Dr.	TU München	7	Immunology Screen
Ollert	Markus	Prof. Dr.	TU München	8	Allergy Screen
Adamski	Jerzy	Prof. Dr.	Helmholtz Zentrum München	9	Steroid Screen
Zimmer	Andreas	Prof. Dr.	Universitätsklinikum Bonn	10	Nociceptive Screen
Schulz	Holger	Prof. Dr.	Helmholtz Zentrum München	11	Lung Function Screen
Beckers	Johannes	PD Dr.	Helmholtz Zentrum München	12	Molecular Phenotyping Screen
Klingenspor	Martin	Prof. Dr.	TU München	13	Energy Metabolism Screen
Katus	Hugo	Prof. Dr.	Universität Heidelberg	14	Cardiovascular Screen
Höfler	Heinz	Prof. Dr.	Helmholtz Zentrum München	15	Pathology Screen
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	16	Data Management
Schughart	Klaus	Prof. Dr.	HZI - Helmholtz-Zentrum für Infektionsforschung	17*	Host Pathogen Interaction Screen
Hrabě de Angelis	Martin	Prof. 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.**	**Titel**
Hoehe	Margret	Dr.	MPI-MG Berlin	1*	MHC-Haplotypen-Sequenzierung

IG Cellular Systems Genomics in Health and Disease

Koordination: PD Dr. Stefan Wiemann

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	1*	Projekt Coordination
Körner	Cindy		DKFZ Heidelberg	2*	Functional Genomic Resources for NGFNplus
Keklikoglou	Ioanna		DKFZ Heidelberg	3*	Cellular Screening Systems
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	4*	Signalling Network analysis
Gavin	Anne-Claude	Dr.	EMBL Heidelberg	5*	TAP - Protein interaction mapping
Pepperkok	Rainer	Dr.	EMBL Heidelberg	6*	Protein and Network dynamics
Korf	Ulrike	Dr.	DKFZ Heidelberg	7*	Quantitative Proteinarrays
Lange	Bodo	PD Dr.	Max-Planck Institut für Molekulare Genetik	8*	Primary Cancer Cell Models
Schneeweiss	Andreas	Prof. Dr.	Uniklinik Heidelberg	9*	Clinical validation
Beissbarth	Tim	Dr.	DKFZ Heidelberg	10*	Pathway reconstruction & modelling
Bender	Christian		DKFZ Heidelberg	11*	Integrated bioinformatics
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	12*	QM & Standards

NGFN Geschäftsstelle

Koordination: Dr. Silke Argo

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Argo	Silke	Dr.	DKFZ Heidelberg	1	Geschäftsstelle des Projektkomitees von NGFN-Plus und NGFN-Transfer im Programm der Medizinischen Genomforschung

KTT

Koordination: Dr. Hubert Müller

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Müller	Hubert	Dr.	Ascenion GmbH	1	Nationales Genomforschungs-netz: KompetenzCenter Technologietransfer (KTT) – Fortführung

IA Entwicklung prophylaktisch wirksamer Anti-Malaria Verbindungen

Koordination: Dr. Birte Sönnichsen

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Sönnichsen	Birte	Dr.	Cenix BioScience GmbH	1*	Anti Malaria Zielgene und Wirkstoffkandidaten
Matuschewski	Kai	Prof. Dr.	Universität Heidelberg	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.**	**Titel**
Schmidt	Marcus	Dr. med.	Universität Mainz	1*	Chemosensitivity determination, clinical data and tumour tissue banking
Gehrmann	Mathias	Dr.	Siemens Medical Solutions Diagnostic GmbH	2*	Identification of gene signatures predicting drug efficacy
Hengstler	Jan Georg	Prof. Dr. med.	Institut für Arbeitsphysiologie an der Technischen Universität Dortmund	3*	Oncoprofile-Kit

IA Heart Failure Therapy

Koordination: Prof. Dr. Markus Hecker

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Hecker	Markus	Prof. Dr.	Universität Heidelberg	2*	Validierung der Decoy Oligodesoxynukleotid-Medikamentenkandidaten in Herzinsuffizienzmodellen
Müller	Oliver J.	PD Dr. med.	Universität Heidelberg	3*	Zellspezifischer Decoy Oligodesoxynukleotid-Transfer ins insuffiziente Herz

IA Metabolomics in Heart Failure as a Novel Diagnostic Tool

Koordination: Prof. Dr. Hugo A. Katus

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**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	Prof. Dr.	Universitätsklinikum Schleswig-Holstein, Campus Kiel	2*	Metabolic Profiling in Mouse Models of Heart Failure (Erster Projektleiter 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.**	**Titel**
Jankowski	Vera	Dr.	Charité – Universitätsmedizin Berlin	1*	Bioanalytik der chronischen Niereninsuffizienz
Jankowski	Joachim	Prof. Dr.	Charité – Universitätsmedizin Berlin	2*	Effekte auf aktivierte Endothelzellen
Herget-Rosenthal	Stefan	PD Dr.	Universitätsklinikum Essen / Universität Duisburg Essen	3*	Patienten und Proben
Herwig	Ralf	Dr.	Max Planck Institut für Molekulare Genetik (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.**	**Titel**
Becker	Karl-Friedrich	Prof. Dr.	Technische Universität München	1*	Proteinlysat Mikroarrayanalyse für uPA und PAI-1 von Formalin-fixierten Brustkrebsgeweben
				2*	HER2-Rezeptor Expression und Signalwege in Brustkrebsgeweben
Porschewski	Peter	Dr.	Qiagen GmbH	3*	Proteomsignaturen in FFPE-Geweben

IA Subgenome Fraktionation for High Throughput Sequencing

Koordination: Dr. med. Benjamin Meder

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Beier	Markus	Dr.	febit AG, Heidelberg	1*	Development of microarrays for sub-genome preparation
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	2*	Cancer Genome Comparisons
Pfeufer	Arne	PD Dr.	TU München	3a*	Cardiomyopathy Re-sequencing
Meder	Benjamin	Dr. med.	Universität Heidelberg	3b*	Cardiomyopathy Re-sequencing
Strom	Tim	PD Dr.	Helmholtzzentrum München	4*	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.**	**Titel**
Korfhage	Christian	Dr.	QIAGEN GmbH	1*	Development and standardization of new WGA and WTA methods
Wichmann	H.-Erich	Prof. Dr. Dr.	Helmholtzzentrum München	2*	Provision of biosamples of different quality to test whole genome and transcriptome amplification techniques.
Klopp	Norman	Dr.	Helmholtzzentrum München	2*	Provision of biosamples of different quality to test whole genome and transcriptome amplification techniques.
Wichmann	H.-Erich	Prof. Dr. Dr.	Helmholtzzentrum München	3*	Transfer of the results to international organisations in the field of biobanking

*completed projects

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Imprint

Imprint:
Layout and realization:
Dr. Silke Argo, Dr. Cornelia Depner, Dr. Tanja Jutzi, Dr. Johanna Lampert, Anita Miehle
Print:
Baier Digitaldruck GmbH, Heidelberg