

*Genetic and Epigenetic Networks
in Cognitive Dysfunction*

ENCODEY

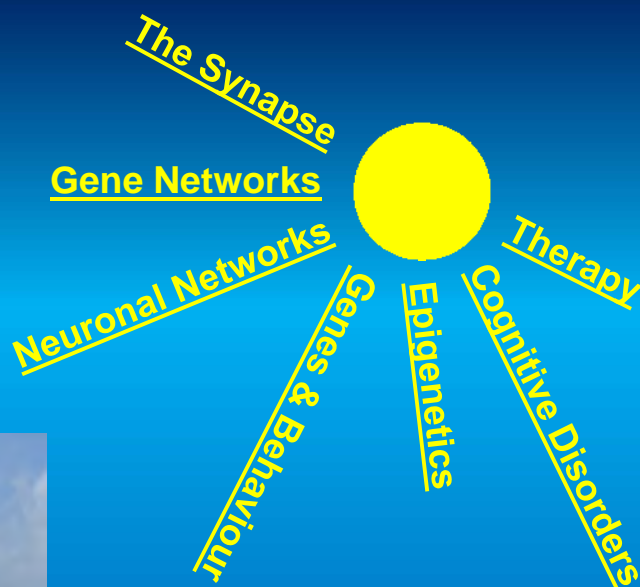
Conference

1st International

Integrative Networks in Intellectual Disabilities

14-17 April, 2013

Coral Beach Hotel ❖ Paphos ❖ Cyprus



Conference book



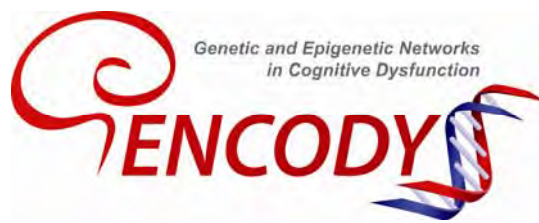
1st INTERNATIONAL GENCODYS CONFERENCE

“Integrative Networks in Intellectual Disabilities”

14-17 April, 2013

Coral Beach Hotel, Paphos, Cyprus

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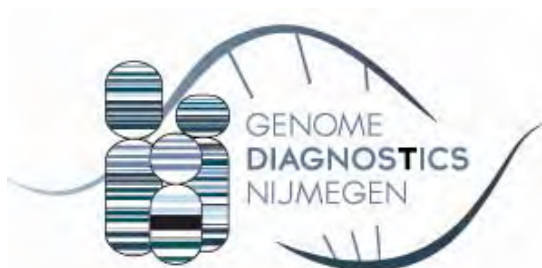
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Genetic and Epigenetic Networks in Cognitive Dysfunction

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WELCOME

We bid you a very warm welcome at the first International GENCODYS Conference. We hope that you will enjoy the scientific contents of this conference as well as its lovely settings, provided by the beautiful island of Cyprus and the hospitality of its people!

The increasing power of sequencing allows the elucidation of causative genetic defects and risk factors in cognitive disorders (CD) by analysis of entire exomes and even complete genomes. A wide variety of chromosomal aberrations and a bewildering number of single gene mutations underlie intellectual disability (ID), and in a growing number of examples share a common etiology with other cognitive defects such as autism and schizophrenia. Elucidation of the complete landscape of all CD-associated genes will allow us to recognize the underlying common pathological mechanisms. Already now, extensive functional interactions are seen between ID-associated proteins and intricate networks are becoming apparent. Examples include protein networks driving synaptic morphology and plasticity and the epigenetic orchestration of neuronal gene expression.

The European funded research consortium GENCODYS exploits a multilevel approach to resolve the integrative networks in intellectual disabilities. We are bringing together top researchers with complementary expertise and patient representatives to apply a systems biology approach to reveal the common molecular and cellular mechanisms leading to cognitive impairment and translational research possibilities. Our overall concept that also will be strongly reflected in the program of our conference is to: (1) Identify novel genes involved in cognitive disorders; (2) Elucidate associated molecular networks that are commonly disrupted in CD; and (3) Identify genetic modifiers and small compounds that are able to modulate the disease phenotype.

Thanks to you we were able to bring together about 110 top researcher, medical doctors and patient representatives, who represent the top in Cognitive Research and related activities. We have 20 invited speakers from the international top including speakers from our own consortium. An additional 18 speakers have been selected on the bases of their recent achievement and submitted abstract. All presentations are related to studies of cognitive dysfunction but in widely varying fields, including genetics, cellular, molecular and physiological studies, genomics and epigenomics and bioinformatics. Integrative network approaches and focus on overlapping disease mechanisms between different disorders are prioritized. Our joined effort and interaction during this meeting will help us broaden our insights in and understanding of Intellectual Disabilities and generate chances to build solid collaborations.

We are glad you chose to participate and share the exciting developments in neurogenetics-driven cognitive research! The future looks bright for Integrative Networks in Cognitive Dysfunction!

Hans van Bokhoven, on behalf of the organizing committee

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GENERAL INFORMATION

Scientific Committee:

- Prof. Hilger Ropers
- Assoc. Prof. Annette Schenck
- Prof. Yann Herault
- Prof. Seth Grant
- Prof. Martijn Huynen
- Assoc. Prof. Frédéric Laumonier
- Assoc. Prof. Vera Kalscheuer
- Prof. Philippos Patsalis
- Prof. Hans van Bokhoven

Organizing Committee:

- Prof. Hans van Bokhoven
- Prof. Philippos Patsalis
- Assoc. Prof. Vera Kalscheuer
- Dr. Dik Hagenbeek

Venue:

Coral Beach Hotel & Resort
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Scientific Program

Sun 14 April 19:00 20:30 **Reception** **and registration desk**

Mon 15 April 8:00 8:50 **Registration**

Morning

8:50 9:00 **Welcome**

Session 1: Building bridges between monogenic and complex cognitive disorders

Chairs: Luciana Musante; Sheikh Riazuddin

9:00 9:30 Keynote Evan Eichler

9:30 9:45 Selected abstract 1 Thomas Arbogast

9:45 10:00 Selected abstract 2 Stephen Meader

10:00 10:30 Keynote Hans-Hilger Ropers

10:30 11:00 **Coffee break**

Session 2: Genes and genetic networks disrupted in Intellectual Disability

Chairs: Sheikh Riazuddin; Luciana Musante

11:00 11:30 Keynote Hans van Bokhoven

11:30 11:45 Selected abstract 3 Vera Kalscheuer

11:45 12:00 Selected abstract 4 Christiane Zweier

12:00 12:15 Selected abstract 5 Tjitske Kleefstra

12:15 12:30 Selected abstract 6 Thomas Wienker

12:30 14:15 **Lunch**

Mon 15 April **Session 3:** Disease mechanisms in Cognitive Disorders: Synaptic plasticity

Afternoon

Chairs: Maksym Kopanitsa, Olivier Dorseuil

14:15 14:45 Keynote Alcino Silva

14:45 15:00 Selected abstract 7 Rhiannon Meredith

15:00 15:15 Selected abstract 8 Barbara Bardoni

15:15 15:45 Keynote Yann Humeau

15:45 16:15 **Coffee break**

Session 4: Molecular mechanisms in Cognitive Disorders: epigenetic mechanisms

Chairs: Annette Schenck, Guillaume Pavlovic

16:15 16:45 Keynote Henk Stunnenberg

16:45 17:00 Selected abstract 9 Nael Nadif Kasri

17:00 17:15 Selected abstract 10 Sofie Metsu

17:15 17:45 Keynote Li-Huei Tsai

Mon 15 April Evening	17:45	19:00	Poster viewing	with drinks
	20:00	21:30	Dinner	
Tue 16 April Morning	Session 5: Molecular mechanisms in Cognitive Disorders: the synapse			
	Chairs:		Guillaume Pavlovic, Annette Schenck	
	8:30	9:00	Keynote	Seth grant
	9:00	9:15	Selected abstract 11	Frederic Laumonnier
	9:15	9:30	Selected abstract 12	Jess Nithianantharajah
	9:30	10:00	Keynote	Stephan Sigris
	10:00	10:30	Coffee break	
	Session 6: Molecular and cellular mechanisms of Cognitive Disorders			
	Chairs:		Martijn Huynen, Caleb Webber	
	10:30	11:00	Keynote	Jamel Chelly
11:00	11:15	Selected abstract 13	Charlotte Kilstrup-Nielsen	
11:15	11:30	Selected abstract 14	Nicoletta Landsberger	
11:30	12:00	Keynote	Guy Rouleau	
Session 7: Genomics in cognition across species				
Chairs:		Caleb Webber, Martijn Huynen		
12:00	12:30	Keynote	Yann Hérault	
12:30	14:00	Lunch		
Tue 16 April Afternoon	14:00	14:30	Keynote	Jonathan Flint
	14:30	15:00	Keynote	Annette Schenck
	15:00	15:30	Keynote	Chris Ponting
	15:30	16:00	Coffee break	
Afternoon/ Evening	16:00	23:30	Social program	including dinner

Wed 17 April	Session 8: Pre-clinical studies towards therapeutic intervention		
Morning	Chairs:	Annick Toutain, Bert de Vries	
8:30	9:00	Keynote	Mara Dierssen
9:00	9:15	Selected abstract 15	Sien Braat
9:15	9:30	Selected abstract 16	Ilaria Meloni
9:30	10:00	Keynote	Ype Elgersma
10:00	10:30	Coffee break	
	Session 9: Diagnostics and therapy: Current practice and future perspective		
	Chairs:	Bert de Vries, Annick Toutain	
10:30	11:00	Keynote	Philippos Patsalis
11:00	11:15	Selected abstract 17	Claire Redin
11:15	11:30	Selected abstract 18	Yoshimi Inaba
11:30	12:00	Keynote	Han Brunner
12:00	12:30	Keynote	Cor Oosterwijk
12:30	14:00	Lunch	End of Conference

KEYNOTE SPEAKERS



Han G. Brunner is full professor and head of the department of Human Genetics at Nijmegen University Hospital. He has initiated and conducted several research projects that use clinical genetic observations as the starting point for human molecular genetics investigations into such topics as intellectual disability, human behaviour, skeletal development, brain development, neuromuscular disease, congenital malformations, and gonadal development and function. Recent key publication: **De novo mutations in human genetic disease.** *Nat Rev Genet.* 2012; 13(8):565-75

Title presentation: **Is intellectual disability mainly a de novo problem?**



Jamel Chelly, MD, PhD, professor at Paris Descartes Medical School - Paris Descartes University Jamel Chelly is a founding member of the European XLMR Consortium that has been instrumental in the remarkable recent progress in the field of X-linked mental retardation and neuronal migration disorders. Current research programs aim to better define and understand disrupted molecular, cellular and neurobiological processes underlying cognitive deficits, neuronal migration defects and malformations of cortical development. Recent key publication: **Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria.** *Nat Genet.* 2009; 41(6):746-52

Title presentation: **Centrosome- and MT-related proteins: the extent of their contributions in the pathogenesis of ID and epilepsy with malformations of cortical development**



Mara Dierssen leads the Neurobehavioral Phenotyping of Mouse Models of Disease research group at the Center for Genomic Regulation in Barcelona. The work of Dierssen has helped to understand the neural plasticity deficits in Down syndrome and establish therapeutic trial. She has demonstrated mechanisms involved in intellectual disability through modifying the activity of brain regions responsible for learning and memory. Dierssen's group has developed novel

methods, and experimental and computational tools in behaviour. Her work establishes a novel paradigm to study the behaviour and cognition in model organisms. The work of Mara Dierssen has received numerous awards and recognitions, including the Sisley-Lejeune Award. Dierssen has a very intense activity in the neuroscience community. She teaches neuroscience at the Pompeu Fabra University Master's Course in Biomedicine, and chaired in 2012 the most important neuroscience forum in Europe in 2012 (FENS Forum). She has been president of the International Behavioral, Neural Genetics Society; she is president elect of the Spanish Neuroscience Society and member of the Executive Committee of the Federation of European Neuroscience Societies. Recent key publication: **Cognitive deficits and associated neurological complications in individuals with Down's syndrome.** *Lancet Neurol.* 2010; 9(6):623-33

Title presentation: **The future of cognitive therapy: can we reset the brain with intellectual disability?**



Evan Eichler, Ph.D., is a Professor and Howard Hughes Medical Institute Investigator in the Department of Genome Sciences, University of Washington School of Medicine. He graduated with a B.Sc. Honours degree in Biology from the University of Saskatchewan, Canada, in 1990. He received his Ph.D. in 1995 from the Department of Molecular and Human Genetics at Baylor College of Medicine, Houston. After a Hollaender postdoctoral fellowship at Lawrence Livermore National Laboratory, he joined the faculty of Case Western Reserve University in 1997 and later the University of Washington in 2004. He was a March of Dimes

Basil O'Connor Scholar (1998-2001), appointed as an HHMI Investigator (2005), awarded an AAAS Fellowship (2006) and the American Society of Human Genetics Curt Stern Award (2008), and elected to the National Academy of Sciences (2012). He is an editor of *Genome Research* and has served on various scientific advisory boards for both NIH and NSF. His research group provided the first genome-wide view of segmental duplications within human and other primate genomes and he is a leader in an effort to identify and sequence normal and disease-causing structural variation in the human genome. The long-term goal of his research is to understand the evolution and mechanisms of recent gene duplication and its relationship to copy number variation and human disease. Recent key publication: **Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations.** *Nature* 2012; 485(7397):246-50

Title presentation: **Neurocognitive Disease and Autism: New Mutations, Genes and Genetic Models**



Ype Elgersma Prof. Dr., Scientific director *ENCORE* expertise center of developmental disorders, Dept. of Neuroscience, Erasmus MC University Medical Center, Rotterdam, The Netherlands. Genetic disorders present us with the unique knowledge of knowing the causal gene and study the impact of the genetic mutation in mouse models of disease. Because of these mouse models, insight in the molecular and cellular basis of the neurological deficits associated with childhood developmental disorders gains rapid progress. In order to be successful in finding a treatment that can ameliorate the neurological deficits, several hurdles must be taken. First, the mouse model must be a good for the disease, and capture its most distinguishing features. Second, the molecular and cellular mechanism that is underlying the disorder must be identified. It must be ensured that the identified mechanism is directly underlying the pathology, and not the result of a homeostatic compensation mechanism. Third, a suitable drug must be found that targets the identified pathological mechanism, and demonstrates reversibility of the affected processes. The fourth and ultimate step is of course to test the toxicity and efficacy of the potential drug in a clinical trial. In this presentation, I will describe basal and clinical research performed at the Dutch *ENCORE* center for neurodevelopmental disorders. Specifically, I will present novel insights in the pathophysiology underlying Neurofibromatosis (NF1) and Tuberous Sclerosis Complex (TSC). I will also discuss how these new findings can be translated to clinical trials. Our work is supported by grants from NWO-ZonMW, the Dutch brain foundation (HsN), the ASF, NINA foundation, ORSA and AFSA patient organizations for Angelman syndrome, and by the Children's Tumor Foundation. Recent key publication: **Treatment of neurodevelopmental disorders in adulthood.** *J. Neurosci.* 2012; 32(41):14074-9

Title presentation: **Molecular mechanisms underlying TSC and NF1: from genes to trials**



Jonathan Flint is a psychiatrist working at the Wellcome Trust Centre for Human Genetics, where he investigates the genetic basis of behaviour. He pioneered the use of outbred mice as a way to identify the molecular basis of complex traits. Outbred mouse populations, derived from fully sequenced progenitors, provide a resource for identifying the genes and sequence variants that contribute to complex phenotypes, including cognition. He is currently running a large project based in China to identify the causes of major depression. Recent key publication: **Sequence-based characterization of structural variation in the mouse genome.** *Nature* 2011; 477(7364):326-9

Title presentation: **Genetic dissection of behavioural variation using outbred mice**



Seth Grant has degrees in physiology, medicine and surgery from the University of Sydney and postdoctoral training at Cold Spring Harbor Laboratory with Douglas Hanahan and later with Eric Kandel at Columbia University. He is currently Professor of Molecular Neuroscience at Edinburgh University and Visiting Professor at Cambridge University and Melbourne University. From 2003-11 he was Principal Investigator at the Wellcome Trust Sanger Institute. He is an elected Fellow of the Royal Society of Edinburgh. Recent Key publication: **Synaptopathies: diseases of the synaptome.** *Curr Opin Neurobiol.* 2012; 22(3):522-9

Title presentation: **Genetic dissection of cognition in mice and humans**



Yann Herval is the Director of the Mouse Clinical Institute (MCI-ICS, Illkirch), and the leader of a research group at the IGBMC (Illkirch). He has a strong interest on gene dosage effect and copy number variation in intellectual disabilities such as in Down syndrome. He is using the mouse as a model organism to better understand the pathophysiology of intellectual disabilities and to propose new therapeutic approaches. Recent Key publication: **Mouse large-scale phenotyping initiatives: overview of the European Mouse Disease Clinic (EUMODIC) and of the Wellcome Trust Sanger Institute Mouse Genetics Project.** *Mamm Genome.* 2012; 23(9-10):600-10

Title presentation: **Mining mouse models to open up new paths for treating Intellectual disabilities**



Yann Humeau is heading a team of neurophysiologists entitled "synapse in cognition" (SynIQ), being part of the interdisciplinary institute for neuroscience (IINS) in Bordeaux, France. Cognitive disorders (CD) mouse models are analyzed using in vivo and in vitro approaches with the aim of understanding the neuronal and synaptic correlates of learning deficits associated with mutations of CD genes. Recent key publication: **Functional roles of synapsin: lessons from invertebrates.** *Semin Cell Dev Biol.* 2011; 22(4):425-33

Title presentation: Pathophysiology of cognitive disorders: **Lessons from multi-scale experimental studies in CD mouse models.**



Cor Oosterwijk is a medical biologist with experience in the field of both molecular and clinical research. Since 2001, he is working as a patient advocate. He is the director of Dutch Genetic Patient Alliance VSOP (director; www.vsop.nl) and secretary general of the European Patients' Network for Medical Research and Health EGAN (www.egan.eu, www.biomedinvo4all.com). He coordinated the FP7 project PatientPartner project concerning stakeholder relationships in clinical research (www.patientpartner-europe.eu). Reference: **Paediatric Clinical research: The Patients' Perspective**. Kent, A, Oosterwijk, C and Poortman, Y, in: Guide to paediatric drug development and clinical research. Eds: Rose & van den Anker, Karger AG, Basel, 2010

Title presentation: **Patient involvement in genetic and clinical research: practical and ethical challenges**



Philippos C. Patsalis is the Chief Executive Medical Director of the Cyprus Institute of Neurology & Genetics and Professor and Director of the Cyprus School of Molecular Medicine. He investigates the genetic cause and mechanisms of genetic diseases and syndromes associated with intellectual disability. Furthermore his research is focused on non-invasive prenatal diagnosis of genetic disorders. Recent key publication: **Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21**. *Nat. Med.* 2011; 17(4):510-3

Title presentation: **Non-Invasive Prenatal Diagnosis of Genetic Disorders**



Chris Ponting is Deputy Director of the MRC Functional Genomics Unit and Professor of Genomics at the University of Oxford, UK. His group undertakes biomedical and evolutionary research using genomics data and methods. He recently led a project that provided an online atlas of transcription for cortical cell layers in adult male mice, and is interested in identifying the evolutionary heritage of different brain regions among diverse avian and mammalian species. His group's evolutionary studies on noncoding RNAs provided the justification required for many that these contribute greatly to biological complexity. Recent key publication: **Evolution and functions of long noncoding RNAs**. *Cell* 2009; 136(4):629-41

Title presentation: **Evolution of brain regions and brain-expressed noncoding RNAs in amniotes**



Hans-Hilger Ropers, MD and Professor of Human Genetics, is currently Director at the Max Planck Institute for Molecular Genetics in Berlin. From 1984-1997 he headed the Department of Human Genetics at the University of Nijmegen, The Netherlands.

Hilger Ropers has a long-standing interest in monogenic disorders with a focus on X-linked and autosomal recessive ID. Recent key publication: **Deep sequencing reveals 50 novel genes for recessive cognitive disorders.** *Nature* 2011; 478:57-63

Title presentation: **Intellectual disability: Genetic dissection of a common disorder.**



Guy Rouleau, MD, PhD, FRCP(C), OQ, Director of the Montreal Neurological Institute and Hospital, Chairholder of the Wilder Penfield Chair in Neuroscience, Full professor in the Department of Neurology at McGill University, Director of the Réseau de Médecine Génétique Appliquée – FRSQ

Over the last 20 years, Dr. Guy Rouleau and his team have focused on identifying the genes causing several neurological and psychiatric diseases, including autism, amyotrophic lateral sclerosis, hereditary neuropathies, epilepsy and schizophrenia, as well as providing a better understanding of the molecular mechanisms that lead to these disease symptoms. Among Dr. Rouleau's main achievements are his contribution to the identification of over 20 disease-causing genes and his discovery of new mutational mechanisms. Dr. Rouleau has published over 500 articles in peer-reviewed journals and has been quoted more than 20 000 times. He has supervised nearly a hundred students at the Masters, PhD and Post-doctoral levels in addition to receiving numerous awards for his contribution to science and society. Recent key publication: **Mutations in DCC cause congenital mirror movements.** *Science* 2010; 328(5978):592

Title presentation: **Neurodevelopmental disorders: Common Mechanisms**



Annette Schenck is heading the *Drosophila* models of brain disorders group at Nijmegen's Human Genetics Department at the Radboud University Medical Centre. Apart from numerous past studies into mechanisms and molecular networks in Intellectual Disability, her group conducts the first large-scale approaches to Intellectual Disability Disorders to systematically map the modular landscape of cognitive genes in health and disease. The goals of her research are to uncover fundamental mechanisms that underlie learning and memory, to integrate *Drosophila* into Next Generation Genome Diagnostics, and to exploit her model and the

identified molecular networks to develop therapeutic strategies to (groups of) cognitive disorders. Recent key publication: **Epigenetic regulation of learning & memory by *Drosophila* EHMT/G9a.** *PLoS Biol.* 2011, 9(1): e1000569

Title presentation: **Neurodevelopmental disorders: Common Mechanisms**



Stephan Sigrist is professor for head of neurogenetics in the biological department of Freie Universität Berlin, and affiliated with NeuroCure cluster of excellence at the Charite Medical Campus. He studies synapses in physiological and pathophysiological context in model systems, particularly *Drosophila*. Genetic analysis is complemented with high-resolution imaging (stimulated emission depletion (STED), biochemical&proteomic analyses as well as physiological methods. Mutations affecting synaptic active zone proteins, associated with autism, are molecularly and functionally characterized. Lately, he started studying mechanisms of synapse

plasticity within age-induced memory impairment. Key publication: **RIM-binding protein, a central part of the active zone, is essential for neurotransmitter release.** *Science* 2011; 334(6062):1565-9

Title presentation: **Polyamines protecting from age-induced memory impairment in an autophagy-dependent manner**



Dr. Alcino Silva's laboratory is studying the biology of learning and memory. His research group is also unraveling mechanisms and developing treatments for learning and memory disorders, such as those associated with Neurofibromatosis type I, and Tuberous Sclerosis. He heads the UCLA Integrative Center for Learning and Memory, and he is a professor in the UCLA Departments of Neurobiology, Psychiatry and Psychology. Dr. Silva is also currently a member of the Board of Regents of the University of Minho, Portugal. In 2002 Dr. Silva founded and became the first President of the Molecular and Cellular Cognition Society, an

international organization with more than 4000 members and with branches in North America, Asia and Europe. Recent key publication: **Modeling hyperactivity: of mice and men.** *Nature Medicine* 2011; 17:541-2

Title presentation: **Reversing neurodevelopmental disorders in adults: from mechanisms to treatments**



Henk Stunnenberg is full professor and head of the department of Molecular Biology, coordinator of the EU FP7 High Impact Project BLUEPRINT and co-chair Steering Board of the International Human Epigenome Consortium, member of EMBO. His research aims at unraveling the molecular basis of development and differentiation emanating from the genome and epigenome in the context of health and disease. Key publication: **The transcriptional and epigenomic foundations of ground state pluripotency.** *Cell* 2012; 149(3):590-604

Title presentation: **Epigenome of Embryonal Stem Cells**



Li-Huei Tsai is the Picower Professor of Neuroscience and the Director of the Picower Institute for Learning & Memory at Massachusetts Institute of Technology. Her lab studies brain development and the cellular and molecular mechanisms that contribute to brain disorders associated with cognitive deficits. Recently, she identified a specific epigenetic pathway that regulates learning and memory and demonstrated that targeting a specific chromatin modifying enzyme can ameliorate cognitive deficits in mouse models of memory disorders. Key publication: **An epigenetic blockade of cognitive functions in the neurodegenerating brain.** *Nature* 2012; 483(7388):222-6

Title presentation: **The role of epigenetic gene regulation in cognitive function and dysfunction**



Hans van Bokhoven is head of the research unit Molecular Neurogenetics at the Radboud University Nijmegen Medical Centre. He investigates the genetic and epigenetic networks that are disrupted in intellectual disabilities, using a multi-level strategy that combines neurogenetics, functional genomics and molecular & cellular neurobiological approaches. Recent key publication: **Genetic and Epigenetic Networks in Intellectual Disabilities**. *Annu Rev Genet.* 2011; 45:81-104

Title presentation: **Genetic & Epigenetic Pathways of Disease**

PRESENTATION ABSTRACTS

1.**Behavioral characterization of 16p11.2 syndrome mouse models**

T. Arbogast¹, A. Ouagazzal¹ and Y. Hérault^{1,2,3}

¹*Institut de Génétique Biologie Moléculaire et Cellulaire (IGBMC), CNRS, INSERM, Université de Strasbourg, UMR7104, UMR964, Illkirch, France*

²*Transgenèse et Archivage Animaux Modèles, TAAM, CNRS, UPS44, Orléans, France;*

³*Institut Clinique de la Souris (ICS), GIE CERBM, Illkirch, France*

Since 2005, genome-wide association studies have identified an important number of copy number variations (CNVs) associated with diseases. Here we interest in a CNV-related human syndromes associated with intellectual disabilities. 16p11.2 syndrome is caused by the recombination of a ~600kb fragment including ~30 genes. Whereas deletion and duplication has been associated with autism and schizophrenia, a reciprocal effect of 16p11.2 gene dosage on BMI and head size has been noted, as deletions are associated with obesity and macrocephaly, whereas duplications are associated with been underweight and microcephaly. The reciprocal impact on BMI and head size for 16p11.2 copy-number variants indicates that some phenotypes could have mirror etiologies depending of changes in transcript levels for genes present in the CNV region.

We have used Cre/Lox technology in targeted meiotic recombination strategy to generate recombination of 16p11.2 mouse homologue regions. We are undergoing the behavioral characterization of mice using two different pipelines. We are also performing cranio-facial analysis and high-fat diet. Comparing deletion and duplication of the two CNV regions, mirror phenotypes have also been observed in mice, but these phenotypes are different from human symptoms and some are even opposites of them.

2.

Large-scale integrative functional analysis of *de novo* CNVs for a cohort of 4000 patients with developmental disorders

Stephen Meader¹, Anneke T. Vulto-van Silfhout², Jayne Y. Hehir-Kwa², Frantisek Honti¹, Rolph Pfundt², Nicole de Leeuw², Bert B.A. de Vries², and Caleb Webber¹

¹MRC Functional Genomics Unit, University of Oxford, Oxford, UK

²Department of Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Copy number variants (CNVs) are a known source of pathogenicity and are thought to contribute to the etiology of a range of congenital disorders, including intellectual disability and developmental delay. However, often patients presenting with similar phenotypic abnormalities possess CNVs occurring at disparate genomic locations, suggesting that the genetic basis of these disorders is heterogeneous and complex.

To understand the contribution of copy number variation to these disorders, we determined the *de novo* CNVs for a cohort of ~5500 patients with congenital abnormalities. *De novo* CNVs were present for 359 of these patients, and disrupt ~3600 different genes. This cohort of patients was deeply phenotyped, with 405 distinct abnormalities annotated from the Human Phenotype Ontology (HPO) across the cohort. For each group of patients presenting with a specific phenotype/abnormality, we looked for functional concordances between the genes disrupted by *de novo* CNVs using a range of functional genomics resources including, mouse-knockout phenotypes, gene ontology, protein-protein interactions, gene co-expression, and our own in house functional linkage network. When considering both deletion and duplication CNVs, we observe at least one statistically significant enrichment or network cluster for 140 phenotypic patient groupings. For 30 HPO patient groups we are able to identify the same genes using convergent methods, while for 50 HPO patient groups enrichment-associated genes were observed to be copy changed in multiple patients. We are replicating these enrichments using novel patients from the DECIPHER database.

Readily-interpretable enrichments were observed for several categories of significant clinical interest. For example, when the orthologues of genes found to be disrupted in patients with seizures are knocked out in mice, the resulting mouse models significantly frequently exhibit seizures phenotypes. Genes affected by CNVs in patients with seizures also clustered with genes that have previously been associated with seizures in a co-expression network. Similarly, recurrently disrupted genes from patients presenting with *severe intellectual disability* are enriched for the mouse phenotypes associated with *abnormal nervous system physiology* and *abnormal synaptic transmission*, among others. This approach identifies novel genes and pathways involved with congenital disorders.

3.

Next-generation sequencing in >450 families with X-linked intellectual disability

Vera M. Kalscheuer¹, Hao Hu¹, Stefan A. Haas², Jamel Chelly^{3,4}, Hilde Van Esch⁵, Martine Raynaud^{6,7,8,9}, Arjan de Brouwer^{10,11}, Tomasz Zemojtel², Guy Froyen^{12,13}, Suzanna G.M. Frints^{14,15}, Frederic Laumonnier^{6,7,8}, Melanie Bienek¹, Corinna Jensen¹, Melanie Hambrock¹, Nicolas Lebrun^{3,4}, Laetitia Castelnau^{3,4}, Marie Shaw¹⁶, Mark A. Corbett¹⁶, Alison Gardner¹⁶, Saffron Willis-Owen^{16,17}, Kathryn L. Friend¹⁶, Stefanie Belet^{18,19}, Melanie Jimenez-Pocquet⁹, Marie-Pierre Moizard^{6,7,8,9}, Nathalie Ronce^{6,7,8,9}, Anna Hackett²⁰, Mike Field²⁰, Eric Haan^{16,21}, Gill Turner²⁰, Bartłomiej Budny²², Magdalena Badura-Stronka²², Anna Latos-Bieleńska²², Peter Wieacker²³, Tjitske Kleefstra¹⁰, Marjolein Willemsen¹⁰, Thomas J. Jentsch^{18,19}, Martin Vingron², Klaus Wrogemann¹, Reinhard Ullmann¹, Thomas F. Wienker¹, Jozef Gecz^{16,21}, Andreas Tzschach¹, Hans van Bokhoven^{10,11}, Wei Chen^{1,18}, Hans-Hilger Ropers¹

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¹¹Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

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¹⁷National Heart & Lung Institute, Imperial College London, UK

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Intellectual disability (ID) is characterized by significantly sub-average cognitive functioning, commonly defined by an IQ lower than 70, and deficits in adaptive behavior. Most severe forms have a single genetic cause, and males are more often affected than females. Therefore, for many years, research has focused on the molecular elucidation of X-linked forms of ID which are thought to account for 10 to 15 percent of all affected males. In excess of >95 XLID genes have been identified, yet mutations in these genes account for only about half of the cases. To advance our understanding of the molecular causes of XLID, we have

employed hybrid capture and massively parallel sequencing to screen X-chromosome exons in probands from >450 unrelated XLID families that had been recruited by the European MRX Consortium and associated groups. ID ranged from mild to severe. By taking into account a wide variety of genetic, bioinformatic, functional and clinical data, we found 15 novel/candidate XLID genes. Many of the corresponding proteins could be grouped into three functional classes, a) synaptic pathways, b) transcriptional and translational regulation and c) proteasome-mediated protein degradation. Our data suggest that mutations in the known and novel XLID genes account for 65-69% of all families with XLID and it is tempting to speculate that most of the missing mutations are not located in the coding regions of the respective genes.

4.

Defects in the genome organizer CTCF cause intellectual disability with microcephaly and growth retardation

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An increasing number of genes involved in chromatin structure and epigenetic regulation has been implicated in a variety of developmental disorders, often including intellectual disability. By trio exome sequencing and subsequent mutational screening we now identified two *de novo* frameshift mutations and one *de novo* missense mutation in the *CTCF* gene in individuals with intellectual disability, microcephaly and growth retardation. Furthermore, a patient with a larger deletion including *CTCF* was identified. CTCF (CCCTC-binding factor) is one of the most important chromatin organizers in vertebrates and is involved in various chromatin regulation processes such as higher order of chromatin organization, enhancer function, and maintenance of three-dimensional chromatin structure. This crucial role in gene regulation prompted us to perform whole transcriptome analyses in blood lymphocytes of three of the patients and eight healthy controls. We found a broad deregulation of genes with a significant overlap between the patients. Down-regulated genes were enriched for genes involved in signal transduction and cell-environment interaction, processes which have been implicated in developmental and cognitive disorders. Together with data from chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) of CTCF in lymphocytes and publicly available ChIA-Pet data of CTCF from the related K562 cell line, we found that CTCF is important for enhancer-driven gene activation and that defects in CTCF affect the genomic interaction of enhancers and their regulated gene promoters, which are required for proper developmental processes.

5.

GATAD2B* loss-of-function mutations cause a recognizable syndrome with intellectual disability and are associated with learning deficits and synaptic undergrowth in *Drosophila

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Background: *GATA zinc finger domain containing 2B (GATAD2B)* encodes a subunit of the MeCP1-Mi-2/Nucleosome Remodeling and Deacetylase (NuRD) complex involved in chromatin modification and regulation of transcription. We recently identified two *de novo* loss of function mutations in *GATAD2B* by whole exome sequencing in two unrelated individuals with severe intellectual disability.

Methods: To identify additional individuals with *GATAD2B* mutations, we performed targeted Sanger sequencing of the *GATAD2B* locus in a selected cohort of 80 individuals based on overlap of the clinical features in the two index cases. To address whether *GATAD2B* is required directly in neurons for cognition and neuronal development, we investigated the role of *Drosophila GATAD2B* orthologue *simjang (simj)* in learning and synaptic connectivity.

Results: A third unrelated patient with *GATAD2B* loss-of-function mutation was identified. Detailed clinical description showed that all three individuals with a *GATAD2B* mutation had a distinctive phenotype with childhood hypotonia, severe intellectual disability, limited speech, tubular shaped nose with broad nasal tip, short philtrum, sparse hair and strabismus. Neuronal knock-down of *Drosophila GATAD2B* orthologue, *simj*, resulted in impaired learning and disrupted synapse morphology.

Conclusion: We hereby define a novel clinically recognizable intellectual disability syndrome caused by loss-of-function of *GATAD2B*. Our results in *Drosophila* suggest that *GATAD2B* is required directly in neurons for normal cognitive performance and synapse development.

6.

De novo truncating mutations in ASXL3 cause a novel clinical phenotype

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Molecular diagnostics can resolve locus heterogeneity underlying clinical phenotypes that may otherwise be co-assigned as a specific syndrome based on shared clinical features, as well as associate phenotypically diverse diseases to a single locus through allelic affinity. Here we describe a novel syndrome which shares characteristics with Bohring-Opitz syndrome (BOS), a disease that is associated with mutations in ASXL1. Using genome wide sequencing we identified heterozygous, de novo truncating mutations in ASXL3, a transcriptional repressor related to ASXL1, in four unrelated probands.

7.

Local connectivity in the Fragile X mouse model & developmental time-windows

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Abnormal brain connectivity is proposed to underlie cognitive and behavioural impairments in many neurodevelopmental disorders (NDDs) of autism and intellectual disability (Belmonte & Bourgeron 2006). At the synaptic level, spine abnormalities are consistently observed in post-mortem brain tissue from both humans and mouse models for specific NDDs. However, the relationship between structural changes at the synapse and functional connectivity has not been fully investigated in these disorders.

We tested whether local functional hyperconnectivity occurs between pyramidal neurons in medial prefrontal cortex of the Fragile X mouse model. We found an increased connectivity between neurons in Fragile X mice at 2-3 weeks postnatal age. Furthermore, these excitatory connections were slower and failed to recover from short-term depression as quickly as their wildtype counterparts. However, these changes were restricted to early postnatal development and disappeared by 4-5 weeks postnatal age.

Many NDDs are characterised by age-dependent symptom onset and regression, particularly during early periods of life. We propose that developmentally-dependent alterations of the synapse, as illustrated in our data in the medial prefrontal cortex and by others in sensory cortex, can be unified into a key concept: namely, time-restricted windows for impaired synaptic phenotypes exist in NDDs, akin to critical periods during neurotypical sensory development in the brain (Meredith et al., 2012). Existence of time-windows has significant implications for our understanding of early brain development in NDDs and may indicate vulnerable periods when the brain is more susceptible to therapeutic treatments.

Belmonte MK & Bourgeron T (2006) Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nat Neurosci*. 9(10):1221-5.

Meredith, Dawitz & Kramvis (2012) Sensitive time windows for susceptibility in neurodevelopmental disorders. *Trends Neurosci* 35:335-44.

8. Is *FMR1* expression regulated via a mRNA/miR-network at the synapse?

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The mutation responsible for Fragile X syndrome (FXS) is an expansion of a CGG repeat located in the 5'UTR of the mRNA of the *FMR1* gene. Two main types of expansions have been described. The full mutation (> 230 CGGs) is abnormally methylated resulting in the inactivation of *FMR1*, while moderate unmethylated expansions (60 - 200 CGGs) are called premutations. The absence of the gene product of *FMR1*, Fragile X Mental Retardation protein (FMRP), causes the clinical features of FXS, which include intellectual disability, autistic behaviour, attention deficits, and some dysmorphic features. In addition, abnormal dendritic spines in the brain of patients as well as in animal models have been reported. FMRP is an RNA-binding protein involved in several steps of RNA metabolism. Most individuals with the premutation have normal intellectual abilities, however some children with the premutation have developmental problems including attention deficit hyperactivity disorder, shyness, social anxiety and autism spectrum disorders. Individuals carrying the premutation may be affected after their 50s by a neurodegenerative disorder, the Fragile X associated Tremor/Ataxia syndrome (FXTAS) developing ataxia, parkinsonism, peripheral neuropathy, essential tremor, progressive memory and executive functions deficits anxiety. The neuropathological hallmark of FXTAS is the presence of ubiquitin-positive intranuclear inclusions in neurons and astroglia, containing the premutated mRNA of *FMR1* whose increased level (2-8 fold) represents the primary cause of this disorder.

In the present study we identified three microRNAs, namely miR-101, miR-129-5p and miR-221, which specifically target the 3'UTR of *FMR1* and can modulate its expression throughout the brain particularly at the synaptic level where their expression level is high. The expression level of miR-221 is reduced in synaptosomal preparations of the young mice model for FXTAS (displaying a 2-fold increased level of *Fmr1* mRNA), where neuronal inclusions are not yet present, suggesting a general deregulation of transcripts located at the synapse in these mice. By transcriptome analysis we show a robust deregulation of the expression levels of genes involved in learning, memory and autistic behavior in these young mice. These data justify the phenotype observed in premutated individuals and confirmed the neurodevelopmental nature of FXTAS. Interestingly, many of those deregulated mRNAs are target of the same miRNAs that modulate the expression of *FMR1* at the synapse. Altogether our data suggest the existence of a mRNA network at the synapse linked by miRNAs that is perturbed by the elevated expression of *FMR1* mRNA.

Patients affected by intellectual disability have been shown to carry a deletion of the Xp112-Xp11.3 region, including miR-221, suggesting that the absence of this miRNA family might contribute to the phenotype of these patients. miR-221 is a target of MeCP2, the gene causing the Rett syndrome and, in addition, its expression was reported to be down-regulated by the activation of mGluRI receptor. The characterization of the role of miR-221 in development and maturation of normal and FXS neurons is in progress and new results will be presented and discussed.

9.

Histone Methylation Dynamics in Synaptic Plasticity

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Alterations in synaptic strength are proposed to underlie learning and memory. Emerging evidence suggests that chromatin remodeling via histone post-translational modifications serves as a critical mechanism to regulate gene transcription required for persistent changes in neuronal activity. The importance of these histone post-translational modifications for learning and memory is further underlined by mutations in various histone methylation and demethylation enzymes that cause cognitive disorders, including intellectual disability (ID) disorders. One of these disorders, Kleefstra syndrome, is a form of ID with autism-like features. The syndrome is caused by haplo-insufficiency of euchromatin histone methyltransferase 1 (EHMT1), which encodes a histone methyltransferase capable of mono- and dimethylation of lysine 9 of histone 3 in euchromatic regions of the genome. Here we investigated the dynamic nature of the epigenetic marks associated with the function of EHMT1. In particular we found that histone methylation is highly dynamic in the phase of synaptic plasticity. When histone methylation was blocked by transgenic intervention or pharmacology, synaptic plasticity was impeded. Furthermore changes in histone methylation were associated with changes in the expression of synaptic proteins. Together these data demonstrate that histone methylation is actively regulated in cortical neurons and is required for the expression of synaptic plasticity.

10.

A DYNAMIC MUTATION AT FRA22A INACTIVATES THE *CSNK1e* GENE IN A PATIENT WITH CIRCADIAN RHYTHM ABNORMALITIES

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Fragile sites of the rare, folate sensitive type represent dynamic expansions of CGG repeats in the genome and have facilitated the identification of several disease genes. For instance, cytogenetic expression of FRAXA on Xq28 led to the identification of *FMR1* as the gene causative of the fragile X syndrome, a common form of intellectual disability. Silencing of the associated gene is caused by methylation of the expanded repeat. Following this discovery, several additional fragile sites have been cloned at the molecular level and in approximately half of the cases the dynamic expansion appears associated with specific cognitive and psychiatric disorders. However, the majority of fragile sites has not yet been characterized at the molecular level, despite the fact that many of those were identified predominantly in patients with cognitive or psychiatric problems. We studied a patient presenting with sleeping problems and recurrent depressive episodes who showed cytogenetic expression of FRA22A, indicative of a dynamic mutation at 22q13.

We identified an expanded CGG repeat in the first coding exon of the *CSNK1e* gene as the molecular basis of this fragile site. This repeat appears highly polymorphic in the normal population with repeat lengths ranging from 7 to about 23 units in 200 control chromosomes. Expansion of over 250 repeats of this *CSNK1e* associated CGG triplet was shown in the proband using Southern blot analysis and repeat primed PCR. Bisulphite sequencing and pyrosequencing technology demonstrated that this expansion is accompanied by hypermethylation of the promoter region. Subsequently, real time RT-qPCR showed reduced *CSNK1e* expression, presumably as a consequence of the promoter methylation in the patient. In control individuals, this promoter region is unmethylated.

CSNK1e is a member of the family of kinase casein kinase I proteins, implicated in the control of cytoplasmic and nuclear processes including DNA replication and repair. Interestingly, *CSNK1e* is a highly conserved gene that encodes for a protein which phosphorylates PERIOD (PER1, PER2 and PER3), an important player in the clock pathway regulating the circadian rhythm. In humans, mutations in *CSNK1e*, its closest homologue *CSNK1d* and the *PER2* gene are found to cause familial advanced sleep phase syndrome (FASPS). In mice, functional loss of *Csnk1e* by removing exon four leads to a minor, but significant increase of the circadian period.

In conclusion we collected evidence that FRA22A lies within the *CSNK1e* gene and that the methylation of the expanded repeat silenced one allele of this gene. Given the importance of this gene in the regulation of circadian rhythm, it is tempting to speculate the mutation plays a role in the sleeping disorder and possibly depression of our patient.

11.

Mutation of the *PTCHD1* gene, which encodes a transmembrane protein expressed in postsynaptic dendritic spines, is associated with non syndromic intellectual disability and autism

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The *PTCHD1* gene (Patched Homology domain 1) has been involved in previous studies describing genomic deletions and missense mutations in patients with autism and/or non-syndromic intellectual disability (ID). Considering that this gene represents a strong candidate for these neurodevelopmental disorders, we searched for mutations on genomic DNA of patients and families collected from the EuroMRX consortium cohort. We characterized the first point deletion in the *PTCHD1* gene coding sequence (c.2128delC, p.L710CfsX12), which would lead to the expression of a truncated form of the PTCHD1 protein lacking the last 180 amino-acids, in a French patient with non-syndromic ID (family T143).

Little is known regarding the neurodevelopmental expression of the *PTCHD1* gene, as well as the neuronal function of the encoded protein. We performed neurodevelopmental expression and neuronal subcellular localization studies in mouse brain.

By RT-qPCR, we found a high expression level in midbrain structures at different fetal stages, and a peak of expression level in the cerebellum and hypothalamus in adult stages. Immunocytochemistry analyses on mature primary neuronal cultures using a custom PTCHD1 antibody revealed that the protein was localized at the plasma membrane and along dendrites. More precisely, PTCHD1 colocalized with postsynaptic markers of inhibitory and excitatory synapses, which was confirmed *in situ* on mouse brain by immunogold labelling. Interestingly, the PTCHD1 mutated construct transfected in hippocampal primary neurons didn't reach synapse and seemed to be maintained in cell body. We showed that the C-terminal tail (40 amino-acids) of PTCHD1 (deleted in T143 family) is essential for dendrite sorting and synaptic localisation.

Lastly, yeast two-hybrid screening on these last 40 amino-acids revealed some interactions with proteins of the postsynaptic density and the NMDA receptor multiprotein complex, linking PTCHD1 to intracellular signaling pathways.

In summary, we confirm the contribution of *PTCHD1* gene mutations in X-linked ID and autism therefore suggesting that this gene would have a crucial role in cognition and communication processes during the development of the central nervous system.

12.

Genetic interaction of disease-relevant genes modulates selective components of cognition in touchscreen assays.J. Nithianantharajah¹, N.H. Komiyama¹ L.M. Saksida², T.J. Bussey² & S.G.N. Grant¹¹Centre for Clinical Brain Sciences and Centre for Neuroregeneration, The University of Edinburgh, Chancellors Building, 47 Little France Crescent, Edinburgh EH16 4SB UK.²Department of Experimental Psychology, University of Cambridge & The MRC and The Wellcome Trust Behavioural and Clinical Neuroscience Institute, University of Cambridge, Cambridge, CB2 3EB UK.

Cognitive deficits are a core feature of most neurological and psychiatric disorders, however unravelling the genetic basis of cognitive disorders is complex due to the involvement and interaction of multiple genes, which manifest in overlapping cognitive impairments. Human genetic studies have elucidated that many of the mutations implicated in cognitive disorders converge upon genes associated with the synapse - the connection between neurons that form the most fundamental information-processing units in the nervous system. Little is known about the genetic basis of distinct aspects of higher cognitive functions such as complex forms of learning and memory, attention and executive functions (including cognitive flexibility and response inhibition). Moreover, there is currently negligible evidence exploring the involvement of epistasis or non-additive gene interactions in the context of cognitive functions. Towards this, using mice with mutations in *Dlg2* and *Magi2*, two key synaptic scaffold genes implicated in cognitive disorders, we examined the role of these genes and gene interactions on different aspects of cognition. Exploiting the emerging technology of the touchscreen assays, a useful behavioural tool for modelling higher cognitive functions in rodents, we observe evidence for complex genetic interactions whereby the *Dlg2* x *Magi2* double mutants either show a *neutral* phenotype (double mutant has an intermediate phenotype between that of the single mutants) or a *genetic suppression* or *enhancement* (double mutant has a less or more severe phenotype than one predicted by the additive effects of the single mutants) in selective cognitive functions. These findings provide novel evidence for gene interactions underlying cognition and will be informative towards elucidating how mutations in multiple susceptibility genes gives rise to distinct and overlapping cognitive phenotypes, and influence disease susceptibility.

13.

CDKL5 affects neuronal polarization through its interaction with Shootin1Sarfraz Nawaz¹, Laura Rusconi¹, Francesco Bedogni², Nicoletta Landsberger^{1,2}, Charlotte Kilstrup-Nielsen¹¹Laboratory of Genetic and Epigenetic Control of Gene Expression, University of Insubria, Busto Arsizio, Italy;²Division of Neuroscience, Rett Syndrome Research Center, San Raffaele Scientific Institute, Milan, Italy.

Mutations in X-linked cyclin-dependent kinase-like 5 (CDKL5) are associated with early-onset epileptic encephalopathies characterized by the onset of intractable epilepsy during the first weeks after birth, severe developmental delay, hypotonia and often the presence of some Rett syndrome-like features. The involvement of CDKL5 in neurodevelopmental disorders and its high expression levels in the maturing brain underscores the importance of this kinase for proper brain development. However, the precise pathogenic mechanisms underlying the disease onset still have to be fully understood. CDKL5 is present in both the nucleus and cytoplasm of cultured cells and neurons and, accordingly, interactors of the protein belong to both compartments. Mice lacking CDKL5 do not show gross alterations in brain structure but the protein has recently been found to be involved in regulating neuronal outgrowth and synapse stability through pathways involving Rac1 and NGL-1, respectively.

With this communication we provide evidence that CDKL5 is involved in regulating neuronal polarization. An essential step during neuronal differentiation is the establishment of a polarized morphology with a single axon and multiple dendrites. This process requires that neuronal symmetry is broken and certain factors accumulate in the distal tip of the axon-to-be. In a yeast two-hybrid screen, using the C-terminal region of the kinase as bait, we identified shootin1, a key regulator of axon outgrowth and neuronal polarization, as a novel interactor of CDKL5. Importantly, we could confirm the interaction between the two endogenous proteins in vivo in mouse brain extracts. Even if CDKL5 and shootin1 have different temporal expression profiles in mouse brains and primary neurons they are both strongly expressed in the period of axon formation and elongation and in young primary hippocampal neurons CDKL5 was found to co-localize with shootin1 in the axonal growth cone. Primary hippocampal neurons depleted for CDKL5 show a defect in neuronal polarization as evidenced by staining with axonal markers. Interestingly, shootin1 phosphorylation is impaired in neurons devoid of CDKL5 suggesting. In the developing brain, neuronal polarization is tightly associated with radial migration of neurons in the cortical plate; interestingly, we find that the knock-down of shootin1 in the neuronal progenitors of mouse brain causes a delay in neuronal migration similarly to that observed for CDKL5. Altogether, these data suggest that CDKL5 regulates neuronal polarity and migration by acting upstream of shootin1 and defects in these intricate processes are likely to be involved in the pathogenesis of CDKL5 associated disorders.

14.

Revealing a novel and unexpected function of MeCP2, possibly involved in RTT pathophysiology

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Dramatic progress has been made in the last 12 years, since the discovery of *MECP2* as the RTT-causing gene, providing insight into the pathogenesis of the disease. Generally, MeCP2 is considered a ubiquitously expressed methyl-DNA binding protein that represses transcription by promoting chromatin compaction. However, recent studies have proposed that MeCP2 might also have activating functions. MeCP2 differential phosphorylation might render it a multifunctional protein. Indeed, even though transcriptional regulation is still considered the main function of MeCP2, other roles have been proposed. One report demonstrated an interaction of MeCP2 with the protein YB1 suggesting an involvement of the methyl-binding protein in alternative splicing, whereas our group has contributed proposing that RTT may be also given by hypofunctional protein synthesis in brain cells.

With this communication, we propose yet another function of this protein. We demonstrate that MeCP2 is a constituent of the centrosome. In fact, by immunofluorescence we have demonstrated that, in cultured cells, MeCP2, and, in particular, one of its specific phosphoisoforms, localizes in the centrosome. Importantly, the centrosome localization is observed also in primary hippocampal and cortical neurons and also by centrosomal fractionation experiments. The presence of MeCP2 in centrosomes and the validity of the used antibodies have been validated by shRNA MeCP2 knock-down or by using *Mecp2*-null neurons. We have further characterized the centrosomal localization of MeCP2 using centrin and pericentrin markers and by microtubule disruption we have demonstrated that this localization is independent of microtubules. Because centrosomal components can affect the microtubule nucleation capacity of the centrosome, we have measured microtubule nucleation in MeCP2-ablated cells and revealed that the knockdown of MeCP2 severely impairs the ability to nucleate cytoplasmic asters. The effect of MeCP2 ablation on cell-cycle dynamics has also been assessed. Interestingly, our results find clear phenotypic effects that seem to be confirmed by analyzing the expression of specific genes during brain maturation.

Even though existing results are contradictory, the centrosome seems not only to provide a structural hub for the microtubule array but appears as the major microtubule nucleation site and is involved in cell proliferation, neuronal polarization, maturation and migration. Therefore, it is not surprising that several permanent or transient components of the centrosome have been associated with diverse neuropsychiatric diseases. Thus, we propose that the novel centrosomal function of MeCP2 and its involvement in cell proliferation might be of relevance for neuronal maturation and differentiation and, therefore, has to be considered in the pathogenesis of Rett syndrome and MECP2-related disorders.

15.

Targeting the GABA(A) Receptor in Fragile X Syndrome: From Molecular and Functional Deficits to Therapeutic Potential

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In previous work, we detected underexpression of several GABA(A) receptor subunits and other components of the GABAergic system in *Fmr1* knockout mice and *dFmr1* deficient *Drosophila melanogaster*. GABA(A) receptors are the main inhibitory neurotransmitter receptors in the mammalian brain and are involved in anxiety, epilepsy, insomnia, depression and learning and memory, processes implicated in fragile X syndrome. It was therefore hypothesized that deficiencies in the GABAergic system could contribute to the clinical features of fragile X patients. In this study we examined possible functional consequences of a reduced GABA(A) receptor expression and tested the therapeutic potential of GABAergic drugs.

The functional properties of GABA(A) receptors were analyzed by means of patch-clamp recordings on hippocampal slices of *Fmr1* knockout mice and wild-type littermates. Whole-cell recordings from CA1 pyramidal neurons revealed a significant decrease ($p < 0.05$, two way RM-ANOVA) of evoked inhibitory postsynaptic currents (IPSCs) in *Fmr1* knockout mice without significant differences in the frequency and amplitude of spontaneous and miniature IPSCs. In addition, we investigated whether reduced inhibitory signalling was accompanied by changes in excitatory transmission in the CA1 region. When analyzing NMDA-dependent long-term depression (LTD), we observed a significant decrease in the late-phase ($F(1,12) = 7.157$, $p < 0.05$, RM-ANOVA).

The GABA(A) receptor pharmacology has been well documented and several drugs that modulate the receptor activity are available for clinical use. In a proof-of-concept study, we have shown that the synthetic neurosteroid ganaxolone, a positive allosteric modulator of GABA(A) receptors, is able to prevent audiogenic seizures in *Fmr1* knockout mice. These experiments were carried out in three-weeks-old mice, while most expression studies were carried out in adolescent mice. Here, we determined the expression levels of relevant GABA(A) receptor subunits at this young age and found significant underexpression in cortex, hippocampus and cerebellum. To further explore the therapeutic effect of ganaxolone in *Fmr1* knockout mice, we evaluated the effect of the drug in the marble burying paradigm. Acute ganaxolone treatment is able to correct the abnormal marble burying behaviour of *Fmr1* knockout mice without having an effect in wild-type littermates ($p < 0.001$, ANOVA).

In conclusion, our results support the idea of a pivotal role of the GABAergic system in the pathogenesis of fragile X syndrome. The patch-clamp results indicate an impairment of the functional properties of hippocampal GABA(A) receptors. Drugs that specifically target these receptors are able to correct several aspects of the mouse phenotype. Our data add further evidence to the hypothesis that the GABA(A) receptor is a promising target for rational therapy for fragile X syndrome.

16.

GluD1, a common altered player in neuronal differentiation from both MECP2-mutated and CDKL5-mutated IPS

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Rett syndrome is a monogenic disease due to de novo mutations in either *MECP2* (classic and Zappella variant) or *CDKL5* genes (early onset seizure variant). In spite of their involvement in the same disease, a functional interaction between the two genes has not been proven. MeCP2 is a transcriptional regulator; *CDKL5* encodes for a kinase protein that might be involved in the regulation of gene expression. Therefore, we hypothesized that the two genes may lead to similar phenotypes by dys-regulating the expression of common genes. To test this hypothesis we used induced pluripotent stem (iPS) cells derived from fibroblasts of one Rett patient mutated in *MECP2* (p.R306C) and 2 patients, one female and one male, with *CDKL5* mutations (p.Q347X and p.T288I). Expression profiling was performed in *CDKL5* mutated cells by microarray technology and interesting genes were confirmed by real-time RT-PCR in both *CDKL5* and *MECP2* mutated cells. The only major change in gene expression common to both *MECP2*-mutated and *CDKL5*-mutated iPS cells was for *GRID1*, encoding for glutamate d1 receptor (GluD1), a member of delta family of ionotropic glutamate receptors. GluD1 does not form AMPA or NMDA glutamate receptors. It acts like an adhesion molecule by linking the postsynaptic and presynaptic compartments and inducing inhibitory or excitatory presynaptic differentiation depending on the specific brain region. Our results demonstrate that *GRID1* gene is down-regulated in both *MECP2*-mutated and *CDKL5*-mutated iPS cells and up-regulated in neuronal precursors, providing the first functional link between the two genes. These data give novel insights into disease pathophysiology and pinpoint to possible targets for new therapeutic approaches.

17.

Targeted High-Throughput Sequencing of 220 genes identify a high proportion of causative mutations in patients with undiagnosed Intellectual disability (ID)

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Background: More than 200 genes have been found mutated in monogenic forms of intellectual disability (ID)/mental retardation (MR), about 100 of them being located on the X chromosome. Half of the known X-linked genes is associated with non or pauci-syndromic forms (NS-ID) while the other half is associated with more syndromic forms (S-ID, i.e. ID associated with defined clinical or metabolic manifestations) with a few non-syndromic cases due to the presence of “milder” mutations (in *RPS6KA3/Rsk2*, *ATRX* or *ARX* for instance) or to incomplete penetrance of specific clinical signs. If there is a rather good coverage for diagnostic demands in patients with evocative syndromic forms, in lesser syndromic patients the diagnostic offer is limited to Fragile-X testing and CGH array analysis. The majority of non-syndromic cases remain therefore undiagnosed.

Method: We designed an exon capture for the high-throughput sequencing of 220 genes clearly involved in ID. We developed and validated pooling strategies to reduce sequencing costs per patient for diagnostic and research applications. We successfully sequenced and analyzed a set of 50 patients with undiagnosed moderate to severe ID, including sporadic and familial cases.

Results: We identified seven (14%) causative mutations (in *MECP2*, *MAOA*, *DYRK1A*, *DMD/DP71*, *RAI1*, *TCF4* and *KDM5C*) and eight (16%) likely causative mutations (in *SRPX2*, *HUWE1*, *IQSEC2*, *ANKRD11*, *SLC9A6*, *NRXN2* and *GRIN1*). By identifying certainly or likely causative mutations in about one third of the patients, we hereby confirm the relevance of such targeted sequencing approach for diagnostic of ID. Indeed, this approach that remains less time and cost consuming than exome sequencing analysis leads to a similar proportion of positive diagnostic results (14% vs 16% in de Ligt et al., NEJM 2012). Moreover, the examination of clinical symptoms in our patients also revealed that some “syndromic genes” such as *TCF4*, *RAI1* or *DMD* can be involved in less syndromic forms of ID and should therefore be screened more systematically in NS- ID patients.

18.

Validation of an innovative, low-cost high-throughput real-time PCR test for early diagnosis of Fragile X syndrome in children with neurodevelopmental disorders of unknown aetiology and in newborn screening

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BACKGROUND: Fragile X syndrome (FXS) is the most common monogenic disorder associated with cognitive and behavioural impairment. It usually results from silencing of the *FMR1* gene through epigenetic modifications linked to a trinucleotide CGG expansion within this gene's 5' UTR. We have recently developed an innovative high-throughput FXS test utilizing proprietary protocol and reagents that examine Fragile X related Element 2 (FREE2). FREE2 is located within intron 1 of the *FMR1* gene, and its methylation is altered in FXS and is related to the type and severity of cognitive impairment. This FREE2 test can be easily adopted by most diagnostic laboratories as it does not require any specialized equipment and can be performed with minimal investment of training and resources.

AIM: The aims of this project was to perform test validation of a recently developed real-time PCR based test targeting the FREE2 region using a large clinically described cohort, and to compare the results of the new test to those of MALDI-TOF MS, which is the reference method.

METHODS: Real-time PCR and MALDI-TOF MS analyses were performed on venous blood and saliva DNA, and in adult and newborn blood spots. The cohort consisted of 189 controls (CGG <40, cytogenetically normal), 118 premutation (PM, CGG 56-170), and 162 full mutation (FM CGG ~200-2,000) males and females, and 93 sex chromosome aneuploidy individuals, with age range between birth and 80 years. Of these 40 PM (CGG 55-200) and 18 FM females were assessed using Wechsler IQ tests. One 3 mm punch per blood spot was available for analyses taken from 46 FM, 13 PM and 55 control males and females.

RESULTS: The real-time PCR approach accurately differentiated carriers of harmful methylated FM alleles from healthy controls and PM carriers with sensitivity and specificity approaching 100%. Comparison of the real-time PCR output with the reference method, using DNA from venous blood, showed correlation coefficient of 0.99; $p < 0.0001$ in all males; the correlation coefficient was 0.91; $p < 0.0001$ in the 'FM/PM mosaic male' subgroup; the

correlation coefficient was 0.75; $p < 0.0001$ in the 'FM only' male subgroup. The correlation coefficient was 0.78; $p < 0.0001$ for all females using DNA from venous blood and 0.77; $p < 0.0001$ in the 'FM only' females subgroup. Using newborn blood spots, FXS affected males and females showed methylation significantly higher ($p < 0.0001$) than their respective controls and PM groups as assessed by both methods. In the group consisting of only FM females, the real-time PCR assay was significantly correlated with full scale IQ, verbal IQ and with performance IQ ($p < 0.005$; $n = 18$). Several verbal subtest scores also showed strong correlations ($p < 0.001$). For 20 FM individuals whose samples could be matched longitudinally between birth and time of consent at < 2 years of age, there was no significant change in methylation as assessed using the real-time PCR method; and in the larger cohort there was no significant relationship found between FREE2 methylation and age. Furthermore, at a specific fluorescence threshold, the real-time PCR test was specific for only methylated FM alleles and could not differentiate individuals with sex chromosome aneuploidies syndrome from female controls using either blood or saliva.

CONCLUSION: The real-time PCR results were highly correlated with the reference method, with high sensitivity, specificity and prognostic value for detection of hypermethylated FM alleles related to cognitive and behavioural impairment in males and females. While further validation in other independent cohorts is required, this real-time PCR test has the potential to provide a time and cost effective prognostic approach enabling clinicians to provide an earlier diagnosis reflecting specific cognitive /behavioural changes, and thus more accurate advice and management of affected children.

POSTER ABSTRACTS

19.

Investigation of genetic causes of intellectual disability in Romanian children using clinical, cytogenetic and array-CGH diagnostic techniques

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Objective: We present the results of our study regarding genetic abnormalities associated with mental retardation in children.

Material and methods: A total of 250 children were studied using a diagnostic protocol based on dysmorphic and clinical assessment. Genetic investigations included karyotype with GTG banding, FISH and array-CGH.

Results: A specific causes for the mental handicap was identified in 72 children (48%). These included a chromosomal abnormality in 52 cases (19,3%), microdeletion syndromes in 36 children (15,3%), recognizable syndromes in 43 cases (13,3%). 40 patients with normal karyotype were selected for array-CGH due to severity of phenotype, while in eight cases array-CGH was used for molecular characterization of cytogenetic abnormalities. The profiles generated served to the identification/refinement of genetic defects in 28 cases. Some of these anomalies are rarely reported, such as: proximal deletion of chromosome 3p, duplication of chromosome 3p26, deletion of chromosome 4p14, proximal deletion of chromosome 8p, inverted duplication of chromosome 12p, duplication of chromosome Xp. In addition, these profiles allowed the mapping of breakpoints, thus supplying valuable information for further analysis and interpretation.

Conclusions: The clinical diagnosis and conventional genetic tests are, still, very important for diagnosis of genetic syndromes. New techniques, like array-CGH, proved, also, their utility in the investigation of the genetic make-up of patients with intellectual disabilities.

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20.

Whole Exome Sequencing in patients with Intellectual Disabilities

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Intellectual disabilities refers to a generalized disorder, characterized by substantial limitations in intellectual, functioning and adaptive behavior, diagnosed before 18 years of age and affecting about 1-3 % of the general population. Genetically, the high heterogeneity and the unexpected great complexity of the genetic basis should be highlighted. From 50-60% of patients with intellectual disability remain undiagnosed. The use of Whole Exome Sequencing in families with some affected members increases the rate of diagnosis and facilitates the identification of new genes. The aims of this study were: to identify mutations in known intellectual disability genes, to identify new genes responsible for intellectual disability, to establish the phenotype-genotype correlation and to provide genetic counseling. We sequenced 32 individuals from eight families of self-reported European ancestry with unexplained moderate to severe intellectual disability using the Illumina HiSeq 2000 Sequencing System. We identified two new mutations in known genes responsible for intellectual disability: the c.2013 mutation delGfs*3 in the *UBE3A* gene and the c.1405C>T p. R469C mutation in the *SMC1A* gene; and one previously described mutation in the *OCRL* gene (c.1567 G>A p.D523N). In the remaining 5 families, several candidate mutations have been identified and more studies are being performed. In conclusion, this study confirms the high heterogeneity and difficulty in the clinical diagnosis of intellectual disability and demonstrates that Whole Exome Sequencing is a very efficient, reliable and cost-effective method which should be incorporated to routine diagnosis in the near future.

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21.

NGS-based parallel analysis of X-linked intellectual disability genes detects causative mutations in a high percentage of sporadic male patients

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Intellectual disability (ID) has a prevalence of 1-3 % and is a major reason for consulting a clinical geneticist. Mutations in X-chromosomal genes are estimated to account for approximately 10 % of male ID patients. Apart from fragile X syndrome, which is at cause in about 25 % of X-linked ID (XLID) and which has been part of the routine diagnostic work-up for many years, more than 90 other XLID genes are known to date. The low prevalence of mutations in each individual gene has, however, rendered routine testing of these genes impractical in patients with unspecific clinical features.

The advent of new sequencing technologies has enabled us to establish a platform combining in-solution enrichment of the coding regions of all XLID genes and subsequent next-generation sequencing (NGS). We have employed this XLID panel for analyzing a cohort of more than 100 unselected male ID patients in whom chromosome aberrations and fragile X syndrome had already been excluded. As a result, we found unambiguously disease-causing mutations in genes such as *MED12*, *CUL4B*, *DLG3*, *SLC9A6* and *UBE2A* in more than 5% of the patients, and variants of unclear pathogenicity were present in additional patients. Considering the high recurrence risks for X-linked disorders, XLID panel analysis has thus been shown to be a valuable diagnostic tool in male patients with non-syndromic or atypical syndromic ID.

22.

Exome sequencing identifies a novel mutation (p.R469C) in *SMC1A* responsible for Cornelia de Lange Syndrome

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Intellectual disability is a genetically heterogeneous condition with a broad clinical phenotype which affects 1-3% of the population where in most of the cases, the cause remains unknown. Exome sequencing has greatly impacted the speed at which new disease genes are identified. Herein, we present a Spanish family with 4 out of 5 male affected with dysmorphic facial features, a profound psychomotor retardation and intellectual disability. Whole-exome sequencing was performed in 2 affected brothers and 2 healthy members and identified 35 delins and 54 single nucleotide variants. Filtering these results based on either autosomic recessive or X-linked inheritance we identified a novel missense c.1405C>T (p.R469C) mutation in exon 9 of the *SMC1A* gene which has been previously associated to Cornelia de Lange Syndrome (CdLS). Direct sequencing for the *SMC1A* gene showed that c.1405C>T variant was present in all affected male members and in any healthy individuals. CdLS results from mutations in the components of the cohesin pathway that mediate cohesion between replicated sister chromatids in dividing cells, in order to ensure proper chromosome segregation. About 60% of CdLS probands have been found to have mutations in the *NIPBL* gene whereas only 5% have mutations in *SMC1A* gene which is associated to a milder CdLS phenotype. To date, all mutations identified in *SMC1A* are missense or small in-frame deletions that preserve the open reading frame of the gene and likely have a dominant-negative effect. We conclude that the application of exome sequencing to the clinical diagnosis of intellectual disability in the near future will reduce the number of idiopathic cases and provide a rich source of sequence variation for the identification of new mutations and genes responsible for intellectual disability.

23.

Epigenetic Mechanisms Underlying Intellectual Disabilities

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While many ID genes have been identified, the etiology is unknown in most affected individuals. Moreover, the functions of most ID genes remain poorly characterized. Evidence is accumulating that control of gene transcription through epigenetic modification of chromatin structure in neurons plays an important role in cognitive processes and in the etiology of ID. However, our understanding of the key molecular players and mechanisms in this process is highly fragmentary. We have recently shown that the Kleefstra Syndrome phenotypic spectrum (KSS) is an ID disorder caused by disruption of several epigenetic regulators, including *EHMT1*, *MLL3*, *MBD5*, and *NR1H3*. Several studies suggest that these genes co-operate in a common biological network. However, it is unclear precisely how this network is composed and how it functions in the regulation of gene transcription in the brain. By the use of RNA interference and P-element mediated mutagenesis, we ablated individual KSS associated gene orthologs in the *Drosophila* brain. Thereafter, we performed mRNA-sequencing to compare the transcriptome of wild type and mutant conditions. We find that there is a strong overlap in gene ontology enrichment of mis-regulated transcripts between different KSS mutant flies. We also find an overlap in differentially expressed genes that is above random. These results, which we will further combine with ChIP-seq data to map the binding sites of KSS proteins, suggest that common molecular pathways are affected by mutations in KSS genes. With this work we are beginning to uncover mechanisms that shape the neuronal chromatin landscape and transcriptome in health and disease.

24.

Whole exome sequencing identifies a missense mutation in *SEPT2* as a probable cause of a new autosomal dominant syndrome with distinctive face, ear anomalies, and learning disability

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Background: Recently, we have presented clinical data of three patients from two unrelated families with a unique combination of clinical features, including prominent eyes and bilateral ptosis. Other features include cleft palate, hearing loss, heart defects, and mild developmental delay. Since the condition is transmitted directly from mother to daughter (family 1) and observed in a male patient (family 2), autosomal dominant inheritance is suggested (Tyshchenko, N. et al. (2011) *Am J Med Genet* 155A (9):2060-2065).

Method: Four members of family 1 (the affected mother, her affected daughter and her healthy parents) were analyzed by whole exome sequencing on a SOLiD4 system.

Results: We identified a heterozygous missense mutation in the *SEPT2* gene. The mutation is present in the two affected individuals and absent in the healthy family members as well as in a large cohort of healthy, unrelated control individuals. According to both polyphen-2 and mutation taster, the mutation is damaging with a probability score of >0.999. The *SEPT2* gene encodes a filament-forming cytoskeletal GTPase. Unlike Ras-type GTP-binding proteins, septins form core oligomeric complexes and the human septin core structure is composed of a linear, non-polar hexamer SEPT7–SEPT6–SEPT2–SEPT2–SEPT6–SEPT7. Sequencing of *SEPT2*, *SEPT6* and *SEPT7*, however, did not yield a plausible mutation in our second family.

Conclusion: Septins have very recently been shown to regulate the collateral branching of axons (Hu et al., *Curr Biol.* 2012 May 16). Since *SEPT2* is also known to act as a scaffold of myosin II, the *SEPT2* gene is a plausible candidate for causing the clinical features of our patients upon mutation. Currently, we are investigating the effect of our *SEPT2* mutation on axon formation in retinoic acid induced SH-SY5Y cells. Since the affected boy of the second family did not show mutations in the septin genes mentioned above, family 2 is currently being sequenced on exome level.

25.

Androgen actions in neurons and gender bias in autism and intellectual disability

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Autism (ASD) and Intellectual Disability (ID) are two common neurodevelopmental disorders with comorbidity and genetic overlap. Another common feature of these two diseases is the existence of a gender bias, very strong for ASD (4 males for 1 female) and notable for the DI (1.4 males: 1 female). Although a large number of genes on the X chromosome are involved in ID and some in ASD, rare and fully penetrant mutations in these genes, if they participate to this male excess, cannot entirely explain it. This sex bias remains unsolved and thus we decided to examine one hypothesis that could explain in part the increased susceptibility of males to neurodevelopmental diseases: the role of prenatal exposure to androgens during male brain development. Several observations suggest indeed the involvement of prenatal androgens in ASD, such as the correlation between prenatal testosterone and traits associated with ASD (works of Baron-Cohen's team) or differential expression of genes involved in the steroid synthesis in ASD individuals. In humans, two waves of early testosterone production occur during the second trimester of *in utero* life and during the first months of the neonatal period. This led to activation and nuclear translocation of the Androgen Receptor (AR), which result in differential expression of genes. It is known that AR is expressed by various cells in the brain and in particular by neurons, and that androgens exert a neuroprotective activity and modulate synaptic density in different brain regions, but little is known about AR target genes in neuronal cells. We are currently investigating the issue of how the effect of androgens in the brain could play a role in the observed sex bias in ASD or DI by analyzing their regulation of gene expression in human neurons. In order to do that, we are analyzing the effect of androgens in neuronal cells, neuronal precursors (NSC) and neurons differentiated from embryonic stem cells (ES), which express AR, by transcriptomic analysis and ChIPseq approach. We are searching if some overlaps exist between the genes/pathways regulated by androgens in neuronal cells and those involved in ASD/DI. We will search for SNPs and rare variants that may affect AR recognition in target genes, and test them for association in patients/controls cohorts. In parallel, we are investigating the non-genomic effects of androgens in neurons, searching for AR co-regulators or for androgen effects on synaptogenic function. Finally, to better understand the role of AR in brain development during the two critical early periods of androgen exposition, we will complete the analysis by looking at the expression in fetal and neonatal human brain, by ISH and/or immunohistochemistry. This study will generate important knowledge on genes and pathways regulated by androgens during human brain development. We hope that it will shed light on molecular mechanisms underlying the sex difference in the susceptibility to neurodevelopmental disorders.

26.

Identification of X-linked genes leading to intellectual disability using exome sequencing

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Background and aim: Intellectual disability (ID) is a common cognitive disorder, with an estimated prevalence of 2-3 % of the worldwide population. Although over 400 genes have been identified, the genetic cause for a significant proportion of the patients suffering from ID is still undetermined. More than 100 genes and 78 nonsyndromic and syndromic intellectual disability loci on the X-chromosome have been associated with ID. Despite systematic, large-scale screens of the X-chromosome, carried out by Sanger sequencing, causative mutations remain unidentified in a substantial number of families with affected members. The aim of this project is to identify novel genetic causes of ID through investigation of families where ID segregates in several generations through exome sequencing.

Material and methods: In the present project 13 families, where ID is likely to segregate as an X-linked recessive trait, will be included. In each family one of the affected individuals will be investigated by exome sequencing. The variations will be investigated using variation databases. Suggestive pathogenic variations in a known or a candidate gene will be verified using Sanger sequencing. Furthermore all the other available affected and unaffected family members will be investigated for the segregation of this mutation.

Results: Preliminary results will be presented at the GENCODYS Conference 2013.

Conclusion: Conclusions will be drawn from the preliminary results and will be presented at the GENCODYS Conference 2013. This study may enable identification of new genes involved in X-linked intellectual disabilities.

27.

Cerebral visual impairment: Differentiating between acquired and genetic causes

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Cerebral visual impairment (CVI) is a disorder in the projection and/or interpretation of the visual input in the brain, leading to visual impairment without major ocular abnormalities. To gain more insight into genetic causes of CVI clinical data of 309 individuals with CVI and a visual acuity ≤ 0.3 were analyzed for etiology, co-morbidity and ocular variables. In this cohort there was a mortality of 5% and co-morbidities were present in almost all individuals: intellectual disability (284/288, 99%), MRI cerebrum abnormalities (174/193, 90%), and deafness (23/187, 12%).

It was possible to identify one or more possible causes for the CVI in 59% (182/309) of the cohort. In 32% (98/309) acquired problems were present, whereas a genetic diagnosis was obtained in 21% (66/309) of the individuals. Chromosomal aberrations were found in half of the individuals with a genetic diagnosis. Some persons had several likely causes, making differentiating between acquired and genetic not always possible. In the genetic group several well-known syndromes could be identified, in which CVI had not been previously reported such as ATR-X, Pitt Hopkins, Coffin-Siris, Mowat-Wilson and Cri-du-Chat syndrome. In the acquired group, specific ophthalmological findings could be observed significantly more frequent than in the genetic group: strabismus (88% versus 62%), pale hypoplastic optic discs (64% versus 26%) and visual field defects (71% versus 29%). We conclude that CVI can be part of a genetic syndrome and that abnormalities in ocular examination are found more frequently in acquired forms.

28.

The Nijmegen Genetics Phenotype Database

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The Nijmegen Genetics Phenotype Database (NGPD) was established in 2002 to collect detailed phenotype data of patients with unexplained intellectual disability and/or congenital anomalies. With the introduction of microarray analysis into clinical practice followed by the introduction of next generation sequencing there is an increasing need for detailed clinical information to interpret the clinical significance of the aberrations detected. The NGPD currently contains detailed clinical data of over 8,000 patients based on the Human Phenotype Ontology (HPO; over 72,500 annotations; median seven per patient). To facilitate submission of patients, we launched an online submission portal, through which clinicians worldwide can enter the clinical data of their patients (www.clinicalfeatures.eu).

We performed hierarchical clustering analysis of the phenotype data using Java and R based programs specifically designed for the HPO to identify patients with overlapping clinical features. For each cluster, an HPO term enrichment analysis was performed using model-based gene set analysis¹. As a proof of principle, we performed clustering analysis of our data, including a well-defined set of patients with the 17q21 microdeletion syndrome (N=85)². Our analysis revealed clustering of the majority of these 17q21 patients. Moreover, a number of other patients were included in the 17q21 cluster, suggesting novel patients with this syndrome. Sanger sequencing of *KANSL1* will be performed in the latter patients.

These results indicate that the NGPD is a rich source for the discovery of patients with overlapping clinical features, ultimately leading to the definition of novel syndromes and identification of novel causative genes.

1. Bauer et al., Nucleic Acids Res, 2010;38(11):3523-32

2. Koolen et al., Nat Genet, 2006;38(9):999-1001

29.

Mouse phenotypes are most informative in the identification of pathways disrupted in complex disordersFrank Honti¹, Stephen Meader¹ and Caleb Webber^{1,2}¹MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford²The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

It is widely thought that the products of genes whose variants are implicated in the same disease are likely to participate in the same biochemical process or pathway. Such pathways are not well defined but their identification could help us understand disease mechanisms and identify common molecular aetiologies that may yield economically viable drug targets.

To identify molecular pathways with relevance to complex disease, we have accumulated diverse data on human genes and their mouse orthologs and integrated these to form a functional-linkage network of human genes. The strengths of links in this network are proportional to the likelihood that the linked genes influence the same phenotypes.

We find that mouse knockout phenotypes are the most useful predictors of human gene–disease associations. We identify clusters in the gene network disrupted by *de novo* mutations in intellectual disability and autism and observe that genes mutated in controls do not form such clusters. We show that the clusters of genes implicated in the same disorder by distinct studies are significantly interconnected and converge on the same pathways.

30.

Lethality, locomotor and cognitive alterations in Ms5Yah mouse model deleted for the App-Runx1 region.T. Arbogast¹ and Y. Herault^{1,2,3}

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Partial monosomies 21 are rare chromosomal anomalies characterized by the loss of a variable segment of the long arm of human chromosome 21 (HSA21). Clinical phenotypes are heterogeneous and range from mild to lethal depending of the affected region. It includes mental retardation, hypertonia, hypotonia, short stature and cranio-facial malformations. Complementary to human genetic approaches, in order to identify dosage-sensitive genes responsive for the symptoms, our team has developed new monosomic mouse models carrying deletions on HSA21 syntenic regions. We are particularly interested in the Ms5Yah mouse model deleted for a 7.7 Mb region from App to Runx1 genes. The deletion results in a important post-natal lethality. Survival Ms5Yah animals are underweight, have a characteristic gait and present impaired performances in rotarod and Morris water maze tests. Whole-genome expression studies confirmed gene dosage effect in Ms5Yah hippocampus. We pinpointed APP as a candidate gene for learning deficits and SMIT-1 as a candidate for post-natal lethality. SMIT-1 gene codes for sodium/myo-inositol cotransporter-1, one of the transporters responsible for importing myo-inositol (MI) into the cells which is essential for the development of peripheral nerve. By giving MI supplement during gestation of mice, we tried to diminish strongly the lethality of Ms5Yah animals. All this data show that Ms5Yah line is as good mouse model of partial monosomies 21 and is perfectly appropriate in the identification of candidate genes responsible for the clinical phenotypes of human patients.

31.

A critical role for the intellectual disability protein OCRL1 in AMPAR trafficking and excitatory synaptic function

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The Lowe Oculocerebrorenal syndrome is a disease caused by mutations in the *OCRL1* gene. One of the symptoms is intellectual disability (ID), which is defined as a global reduction in cognitive and intellectual abilities leading to major deficits in learning and memory. The gene encoding human OCRL1 lies across 24 exons on the X-chromosome. Tissue-specific alternative splicing gives rise to two isoforms, termed a and b. In the brain, only OCRL1a isoform is expressed. Although intensive research over the past years has begun to shed light onto the cellular function of *OCRL1*, it is not understood how alterations in OCRL1 signaling result in changes in the brain. Here, we report the functional characterization of OCRL1a in the brain. In neurons, OCRL1a is expressed throughout the Golgi apparatus and the endosomal system, in accordance with previous studies in non-neuronal cells. Specifically, OCRL1 is present in both pre- and post-synaptic compartments and endocytic clathrin-coated pits. Overexpression of *OCRL1a* in hippocampal neurons reduces the number of excitatory synapses and dramatically decreases the frequency and amplitude of AMPAR-mediated miniature excitatory postsynaptic currents (mEPSC). In contrast, decrease of *OCRL1a* signalling enhances AMPAR-mediated mEPSC without changing the number of excitatory synapses. Furthermore, post-synaptic loss of *OCRL1a* promotes surface expression of GluR2 subunit of AMPA receptor. These results imply that normal activity-driven glutamatergic synapse development is impaired by perturbation of OCRL1a function. Thus, our findings link genetic deficits in OCRL1 to glutamatergic dysfunction and suggest that defects in early circuitry development are an important contributory factor to this form of intellectual disability.

32.

Mutations in the ACTB gene cause a phenotype consistent with severe Baraitser-Winter syndrome.

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Background: Mutations in *ACTB* and *ACTG1* genes have recently been reported to cause Baraitser-Winter syndrome (BRWS) – a rare condition characterized by congenital ptosis, ocular colobomata, anterior neuronal migration disorder (pachygyria, lissencephaly), distinct facial minor anomalies, and intellectual disability. One of the patients carrying an *ACTB* mutation was previously diagnosed with Fryns-Aftimos syndrome (FAS). The main clinical features of FAS are craniosynostosis, anterior pachygyria and cerebral atrophy, a short webbed neck, limited extension of the joints with pterygia and very specific facial features: arched eyebrows; proptosis; hypertelorism; downslanting palpebral fissures; a broad nasal bridge; macrostomia and dysplastic low set ears.

It was recently suggested that BRWS and FAS are in fact the same condition, and the differences observed in both instances are due to age. However, other studies have countered that FAS and BRWS patients' phenotypes are too different to be one and the same disorder.

ACTB and *ACTG1* code for the beta- and gamma isoforms of actin - the nearly identical highly conserved proteins that differ only by four amino acids.

Results: Patient 1 (20 years) exhibits craniosynostosis, anterior pachygyria and subependymal nodular heterotopia; bilateral eye colobomas; intestinal malrotation; ectopic kidneys; diastasis recti; preaxial polydactyly; a short and webbed neck; webbing at the axilla and elbows; and extension limitation of the shoulders, elbows, and knees. Moreover she presents with a striking and distinct pattern of facial anomalies: high arched eyebrows; proptosis; hypertelorism; downslanted palpebral fissures; broad nasal bridge; broad nasal tip and columella; antverted nostrils; macrostomia; very high and narrow palate; crowded teeth; low set, long, dysplastic ears; and a low posterior hairline. The patient has severe ID with lack of speech development.

Patient 2 (8 years) shows heart defects, a bilateral cleft lip, cleft palate, anterior pachygyria, trigonocephaly, a short and webbed neck, axillary webbing and a narrow thorax. Her facial anomalies include high arched eyebrows, hypertelorism, downslanted palpebral fissures, broad nasal bridge, broad nasal tip and columella, antverted nostrils, macrostomia, low posterior hairline. The patient has moderate to severe ID with no speech development.

High resolution array CGH analysis revealed normal results in both patients.

Sanger sequencing of *ACTB* identified the heterozygous missense mutations NM_001101.3:c.359C>T (p.(Thr120Ile) in patient 1 and NM_001101.3:c.220G>A (p.(Gly74Ser) in patient 2. Neither of these mutations has been reported before, not in BRWS patients nor in association with other phenotypes. These mutations are also not listed in dbSNP or NHLBI ESP Exome Variant Server.

The *ACTB* mutation NM_001101.3:c.359C>T in patient 1 is analogous to the *ACTG1* mutation NM_001614.1:c.359C>T previously reported in a patient with BRWS (significantly milder affected than patient 1).

Conclusions: We propose that FAS is a distinct entity with an early-onset, severe phenotype caused by mutations in *ACTB*.

Despite the structural similarity of beta- and gamma-actins and their expression in the same tissues, mutations in *ACTB* cause a distinctly more severe phenotype. This confirms the significant difference in the function of the non-muscular actins during development.

Detailed clinical follow up with genotype-phenotype correlation is elaborated on.

33.

Identification of genes associated with intellectual disability using knockout mice

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One of the most prevalent and severe cognitive disorders is intellectual disability (ID). It affects 1-3% of the population and yet despite its high prevalence, ID is also one of the least understood of all health problems. It is estimated that genetic mutations account for half of the currently undiagnosed cases, and despite recent successes in identifying some of the mutations responsible, it has been suggested that up to 1,000 more genes remain to be identified. To address this, we take advantage of the International Knockout Mouse Consortium (IKMC), a massive investment that generates a resource of knockout mice in order to investigate the function of all protein-coding genes. In this study, our general aim is to use brain samples derived from the IKMC and search for abnormal morphology of the mouse brain in order to identify genes associated with intellectual disability. We have two general approaches; the first is a genome-wide approach. Our preliminary data has already yielded success with the identification of eight genes including *Chd7* (Chromodomain Helicase Dna binding protein 7) and *Wdr47* (WD repeat domain 47), both associated with corpus callosum agenesis, and *Ube3b* associated with microcephaly. The second approach is to apply these mouse knockout resources to well-defined genetic intervals associated with syndromes involving intellectual disability such as the 16p11.2 (OMIM #611913) and the 17q21.31 (OMIM #610443) micro-deletion syndromes. It is important to dissect the role of each gene in these intervals and understand the mechanisms by which these large deletions cause the clinical features because it will suggest avenues for therapy. Both approaches offer complementary resources to human genetic studies.

34

Clinical characterization of six patients with 15q13.2-q13.3 microdeletion

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Individuals with 15q13.3 microdeletion may have wide range of clinical manifestations including intellectual disability (ID), speech delay, epilepsy, autism and schizophrenia. Deletion of *CHRNA7* gene in this region is causative for the majority of neurodevelopmental phenotypes in the 15q13.3 microdeletion syndrome. Subsets of persons with the deletion have no obvious clinical findings at all. During 2009-2012 the chromosomal microarray analysis (CMA) was performed in 1188 individuals due to their clinical indications. In six individuals 15q13.3 microdeletion was found: all of them had typical ~1.5Mb deletion between BP4 and BP5. Here we present the clinical features of six patients with 15q13.2-q13.3 microdeletion.

All our patients (aged 4-8, among them two pairs of sibs) had ID (borderline to severe), speech delay and mild facial dysmorphism but normal growth parameters. Abnormal EEG was found in four out of five tested patients (80%), which is more frequent than previously reported. Still, only one boy has severe treatment resistant generalized epilepsy. Positive family history for epilepsy was documented in two families though. Therefore, according to previous reports it is likely that their epilepsy may be caused by 15q13.3 microdeletion. Aggressive behavior is apparent in two of our patients (33%). Testing of family members has shown familial deletions in all but one patient whose father is unreachable for testing. 15q13.3 microdeletion is one of the most common microdeletions found by CMA in individuals with ID. In our cohort of patients who were tested with CMA it was detected in 0.5%.

35.

Intellectual disability associated with spastic paraplegia and glaucoma in an Algerian consanguineous family maps to 10q23.1-q25.2

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Intellectual disability (ID) and spastic paraplegia (SPG) are disorders with marked clinical and genetic heterogeneity. In both groups, non-specific and syndromic forms have been described. The association of ID and SPG is frequent with more than 80 entries in the OMIM database, but the association of ID, SPG and glaucoma has been reported only twice in the literature: in 4 patients of both sexes in two sibships of a large inbred Swedish pedigree, and in three male Canadian siblings born to first-cousin parents. We had the opportunity to examine three brothers, born of Algerian consanguineous parents, with the same condition. The three patients had mild/moderate ID with normal OFC, associated with non or slowly progressive SPG diagnosed in infancy and no other neurological signs, and juvenile open angle glaucoma. Glaucoma usually remained undetected for years, being diagnosed in adolescence or early adulthood, and was therefore responsible for a severe visual impairment. Brain MRI, metabolic investigations and chromosome analysis were normal. Linkage to the genes *ARX*, *XNP*, *PLP* and *L1CAM* was excluded in our family and sequencing of *MECP2* failed to detect a causative mutation. In addition we have excluded a contiguous gene syndrome by array-CGH analysis (Agilent CGH 1M) which did not detect any CNV. The genetic basis of this rare syndromic form of ID/SPG is still unknown. Consanguinity in all three pedigrees and affected females in one of them support autosomal recessive transmission (OMIM 270850).

By homozygosity mapping in our family we have defined a region of localization of 30.6 Mb containing around 250 genes and MiRNAs at 10q23.1-q25.2 with a maximum Lod Score of 2.53. No gene responsible for ID and/or SPG and/or glaucoma is known in this region. However, in 2009 Dursun *et al.* have reported a consanguineous Turkish family with 5 individuals affected with an autosomal recessive form of SPG (SPG45; OMIM 613162). In addition all patients had ID and 3 had ocular signs with optic atrophy in one. By genomewide linkage analysis the disease was mapped to a 4.6 Mb region containing 87 genes at 10q24.3-q25.1 (maximum 2-point lod score of 3.45 at D10S1710). This interval is included in the region of localization defined in our family. Exome sequencing is pending.

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36.

GABAergic Transmission in the Developing Hippocampus of Fmr1-KO MiceI. Kramvis^{1,2}, M. Sierksma¹, H. Mansvelder¹, R. Meredith¹

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Abnormal neurocircuit function due to altered GABAergic transmission is believed to underlie neurodevelopmental disorders including Fragile X Syndrome (FXS). In the FXS mouse model (Fmr1-KO), GABA_A receptor subunits are down-regulated in the hippocampus. In addition, KO mice exhibit impaired hippocampal-dependent spatial memory and are more seizure-prone. However, it is not yet clear how changes in GABA_A subunit expression affect the functional development of inhibitory transmission in the hippocampus. The aim of this study was to determine whether changes in GABA_A receptor subunit expression in KO mice are reflected at the functional level in developing hippocampus.

Spontaneous phasic inhibitory postsynaptic currents (sIPSCs) and tonic inhibitory currents were recorded from CA1 pyramidal and dentate granule (DG) neurons in brain slices at 2-3 and 3-4 postnatal weeks. For CA1 pyramidal neurons, developmental maturation of phasic and tonic currents was comparable between WT and KO mice at both age groups. In the DG of KO mice a transient increase in decay time normalizes with age, whereas a significant increase in the sIPSC frequency is observed with age. Finally a trend for reduced tonic currents in the DG was observed at the later time point.

Our findings point toward an increased hippocampal phasic inhibition in Fmr1-KO mice during early adolescence despite reduced GABA_A subunit expression. In line however with the reduction of the δ -subunit is the reduction in the DG tonic currents. We will now employ subunit specific pharmacology to challenge and dissect subunit-specific mediated currents as well as expand our analysis to later time points.

37.

Diagnostic routing for exome sequencing in intellectual disability

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Because of the high heterogeneity in intellectual disability (ID), whole exome sequencing (WES) is more cost effective and has a higher diagnostic yield compared to Sanger sequencing. In our routine diagnostic WES analysis pipeline, both the patient's DNA and the DNA of the healthy parents is sequenced. Analysis of variants in approximately 500 ID genes as well as an additional *de novo* analysis is performed. The results are combined in a single overview of potential mutations in known ID genes as well as probable *de novo* candidates. After pre-selection of the most likely disease causing variant(s), Sanger sequencing is performed in order to confirm the presence of the (*de novo*) variant.

In cases with no mutations in the known ID genes, the most likely pathogenic *de novo* mutations were succeeded. Evidence for pathogenicity was mainly based on described gene function, and classification of the variant by *in silico* prediction programs. For each patient, a diagnostic report was written based on the mutation(s) identified and the(ir) clinical relevance, thereby providing a statement whether or not the genetic cause for ID was identified.

The diagnostic routing for ID, as well as the classification of variants, and examples of reports will be presented.

38.

Functional role of microRNA-137 dysregulation relevant to Intellectual Disability

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Intellectual disabilities (IDs) are characterized by significant limitations in intellectual functioning and in adaptive behaviours. Recently, patients with mild ID were identified with chromosome 1p21.3 microdeletions comprising microRNA (MIR)137 thereby causing haploinsufficiency of miR-137. MiRs are short non-coding RNAs that post-transcriptionally fine-tune protein expression by binding to cognate mRNA targets. Recent findings support the notion that cognitive deficits observed in human ID patients may be attributed to loss of dendritic spines or changes in synaptic morphologies. To examine the effect of miR-137 loss-of-function on synaptic development and morphologies in vitro, we used a lentivirus expressing a sequestration vector called 'sponge' containing four miR-137 binding sites. Specificity and efficacy of endogenous miR-137 suppression was confirmed for this sponge. We found that sequestration of miR-137 using this sponge-based strategy in mature hippocampal neurons increased spine density and produced more mushroom shaped spines. Notably, different forms of ID have specifically been linked to defective metabotropic glutamate induced-long term depression (mGluR-LTD), a form of protein synthesis-dependent synaptic plasticity. We hypothesized that miR-137 contributes to synaptic protein synthesis in light of mGluR-induced plasticity. Experiments in organotypic hippocampal slices undergoing mGluR-LTD by applying the mGluR group I agonist 3,5-dihydroxyphenylglycine (DHPG), show an upregulation of miR-137. More specifically, the increase in miR-137 appeared to be mGluR5 rather than mGluR1 dependent, since chemical inhibition of the mGluR5 receptor blocked the LTD-induced miR-137 elevation. To answer the question if miR-137 is necessary for expression of LTD in the CA1/CA3 region of the hippocampus, electrophysiological approaches were used in which LTD was induced using paired pulse low frequency stimulation. Inhibition of miR-137 during the LTD protocol by the introduction of a miR-137 inhibiting oligonucleotide via the patch pipette blocked the LTD expression.

In conclusion, the synaptically enriched miR-137 is involved in spine development and proper functioning of the synapse. Future studies shall address that miR-137 is not only necessary but also sufficient for mGluR-LTD, and will identify the precise molecular mechanism through which this miR acts during synaptic plasticity. These goals will also be achieved by using a conditional miR-137 knock-out mouse enabling us to investigate the role of miR-137 in cognitive deficits associated with ID.

39.

A plethora of novel genes for autosomal recessive intellectual disability

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Recently we have combined homozygosity mapping, targeted exon enrichment and next generation sequencing (NGS) to search for the underlying gene defects in 136 consanguineous families (Najmabadi et al., 2011). In 78 of these families, apparently causative mutations were identified, involving 22 known genes for ARID or related neurological disorders as well as 50 novel candidate genes. These findings confirmed our previous conclusion (Najmabadi et al, 2007) that ARID is extremely heterogeneous.

Since this paper appeared more than 60% of the novel candidate genes could be confirmed by studying mouse and/or drosophila models, or by identifying additional families with allelic mutations. Several of these results were borne out from an even larger study comprising 167 additional Iranian ARID families, involving genotyping, targeted exon or whole exome enrichment and NGS. So far, Sanger validation has been completed for 68 of these. In 56 families, one or more apparently disease-causing variants were found, and two families carried large deletions.

Only seven of the ID genes, identified in our previous study i.e. *ACBD6*, *AP4M1*, *CAPN10*, *LARP7*, *L2HGDH*, *SRD5A3* and *TRMT1*, were also found to be mutated in the second cohort, substantiating our earlier conclusion that ARID may result from mutations in many hundred or even thousands of different genes.

40.

X-CHROMOSOME MICROIMBALANCES IN BOYS WITH INTELLECTUAL DISABILITY AND MATERNAL COMPLETELY SKEWED X-INACTIVATION

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Extremely skewed X-chromosome inactivation (XCI) is a consistent feature of women who carry microscopically detectable unbalanced structural abnormalities of the X-chromosome. It is widely accepted that this deviation from randomness is the result of growth disadvantage of cells in which the active X carries the structural abnormality. We evaluated the frequency of X-chromosome inherited microimbalances in boys with syndromic moderate to severe intellectual disability (ID), whose clinically normal mothers showed completely skewed X-inactivation (100:0 inactivation ratio), based on the methylation status of the *AR* alleles in blood cells. Comparative genomic hybridisation was performed on X-chromosome 44K (Agilent) or 105K (OGT) arrays. Fifteen out of 133 mothers of boys presenting with nonfamilial ID, and five out of 25 mothers of two or more boys with ID had completely skewed XCI. This frequency (~13%) is much higher than that found in adult women from the general population (~1%). The only causative microimbalance detected among the 20 probands with ID was a ~460 kb duplication encompassing *MECP2* in two sibs (5%). Among nonfamilial cases, an inherited ~145 kb microduplication was detected that was found to be polymorphic (DGV, variation_68061), and a ~82.4 kb microdeletion disrupting *SLC25A43* was also carried by the clinically normal brother of a proband. The maternal skewing of XCI is, therefore, unlikely to be related to these two imbalances. Considering that X-chromosome microdeletions/microduplications have been reported in about 10% of familial X-linked ID, the findings in this small cohort of patients suggest that completely skewed XCI in mothers of boys with ID does not enrich the sample for causative X-chromosome microimbalances.

41.

Mutations in *WARS2* and *SARS* are associated with intellectual disability

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Aminoacyl-tRNA synthetases (ARSs) are essential and ubiquitously expressed enzymes responsible for ligating amino acids to cognate tRNA molecules in mitochondria and in the cytosol. Mutations in five mitochondrial ARS have been associated with brain-specific phenotypes, and four genes encoding cytoplasmic ARSs have been implicated in inherited peripheral neuropathy with an axonal pathology.

We report here on two consanguineous Iranian families affected by autosomal-recessive intellectual disability (ARID). Using a combination of homozygosity mapping followed by next-generation sequencing and Sanger sequencing, we have identified mutations in two different ARS genes. In the first family, defects involving the mitochondrial tryptophanyl-tRNA synthetase gene (*WARS2*) co-segregated with moderate ID and athetosis. A frameshift mutation which leads to a premature stop codon was detected in exon 2. Given its location relative to the terminal exon 6, it is likely to cause NMD. The second mutation is a missense change located in exon 1, affecting a predicted mitochondrial signal peptide. Preliminary immunofluorescence studies in human HEK239T cells revealed that the *WARS2* is defective in mitochondrial localization and shows diffuse localization in cytoplasm.

In the second family, a homozygous missense mutation in *SARS*, the cytoplasmic seryl-tRNA synthetase, co-segregated with moderate ID and borderline microcephaly. The change affects an amino acid residue that is highly conserved across the animal kingdom and it leads to unstable protein expression in mammalian cells. Moreover, the missense change leads to reduced enzymatic activity *in vitro*.

WARS2 and *SARS* add to the list ubiquitously expressed genes implicated in ARID. Furthermore, our results underscore the importance of ARSs in neuronal function and brain development.

42.

THE ICS -INSTITUT CLINIQUE DE LA SOURIS- GENETIC ENGINEERING and MODEL VALIDATION DEPARTMENT: GENERATION OF CUSTOMIZED AND READ-TO-USE GENETICALLY ENGINEERED MICE TO UNDERSTAND INTELLECTUAL DISABILITIES

Guillaume Pavlovic, Marie-Christine Birling, Marie Wattenhofer-Donze, Sylvie Jacquot and Yann Hérault

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The Institut Clinique de la Souris – ICS- is a research infrastructure that provides extensive services ranging from the development of mouse models to comprehensive phenotyping. The Genetic Engineering and Model validation Department is dedicated to the development and molecular validation of new mouse models.

Our mission is to help the broad scientific community to improve the knowledge of the genome by understanding the function of protein coding genes and non-coding sequences in the mouse, to identify and understand the genome modifications that lead to diseases and to provide support to develop new treatments and improve the drug discovery process.

Mouse is a very valuable model to better understand the molecular processes of intellectual disabilities (ID) and allows a large panel of genetic modification to mimic these diseases. We can for example generate mutant models to study monogenic linked ID or more complex duplications and deletions of defined genomic fragment (CNVs). Inactivation of brain specific cell types (generation of time and cell specific knock-out) is also possible by breeding conditional knock-out mice with a cre or creERT2 promoter-driven line.

Since 2002, we have generated more than 1000 genetically engineered mouse models for the academic scientific community and for international consortiums (EUCOMM, EUCOMMtools, GENCODYS, IMPC, PHENOMIN). More than 170 publications have already arisen from mice generated at ICS.

We are also driving several internal R&D programs to provide new tools for the scientists as a CreERT2 zoo for in-depth studies (<http://www.ics-mci.fr/mousecre/>), cre and FlpO deleter mice to ease the production of mutant models, TALE/ZFN nucleases for target other mouse genetic backgrounds, ...

43.

Circadian regulations of hippocampal genes and role of Ophn1, an intellectual disability geneJ. Renaud¹, F. Dumont¹, S. Jacques¹, J. Chelly¹, P. Billuart¹ and O. Dorseuil¹¹*Institut Cochin, INSERM U.1016, CNRS UMR.8104, Université Paris Descartes, Paris, France*

Circadian rhythm governs many aspects of behavior and physiology, including the sleep/wake phases. Children with intellectual disability (ID) or autism spectrum disorders (ASD), tend to have modified cycles of sleep, suggesting a dysregulated circadian clock in these pathologies. Interestingly, several synaptic proteins exhibit a circadian expression, suggesting a circadian regulation of synaptic activity by the master clock.

We have investigated the apparent interplay between circadian clock and synapse activity by transcriptomic analysis of gene expression in wild type mouse hippocampus over a 24h period. This strategy allowed us to define a list of genes with a circadian expression in hippocampus. Pathway analyses on function of these genes show that not only the master clock genes follow a circadian expression, but also the ubiquitination and glutamate receptor signaling. Surprisingly, we show that ID/ASD genes and synaptic genes do not appear enriched in our circadian gene list, thus not supporting a circadian dysregulation in hippocampus as a major pathogenic mechanism of ID/ASD.

We recently reported that Oligophrenin1 (Ophn1), an X-linked ID gene product regulating synaptic activity through glutamate receptor internalization, interacts with Nr1d1, a transcriptional repressor functioning in master clock negative loop. To search for circadian dysregulations in Ophn1-KO mice, we compared gene expression patterns of WT and KO in the hippocampus over a 24h period. We report here, slight dysregulations of numerous circadian-regulated genes, including some master clock genes, as well as genes of the protein ubiquitination pathway, glucocorticoid receptor signaling and regulation of transcription. Promoter analysis of the dysregulated circadian genes shows some enrichment in Nr1d1-target genes, thus highlighting the potential role of Ophn1-Nr1d1 complex in pathophysiology of the disease. Altogether this work illustrates possible involvement of Ophn1, through its interaction with Nr1d1, in the control of gene expression in hippocampus.

44.

ID genes and synaptopathy

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Recent discoveries on the genetic causes of intellectual disability (ID) or autism spectrum disorders (ASD) let appear that numerous ID/ASD genes encode i/ proteins directly observed at synapses or ii/ proteins located in the nucleus. During brain development, memory acquisition and learning, hippocampal neurons exhibit formation of highly complex and dynamic connectivity with up to thousands synapses per neuron. Conversely, these contacts appear altered in some ID or ASD situations of human pathology and in several mouse models of ID/ASD, suggesting a close association between ID/ASD and “synaptopathy”.

Pathology of synapses may therefore be considered as a hallmark of cognitive defects. To investigate this hypothesis, we study new ID genes discovered through the “Gencodys” network by systematic characterization of their ability to trigger “synaptopathy in a culture dish”. Primary culture of mouse hippocampal neurons are differentiated in vitro and morphologically analyzed after down-regulating expression of each ID gene by RNA-interference technology. Potential defects at early and late stages of differentiation are evaluated by measuring neuritogenesis (dendritogenesis, axonogenesis) as well as synaptogenesis using fluorescent microscopy approaches. In parallel, correlations are searched for each ID gene between their phenotypes and its expression pattern during in vitro and in vivo neuronal differentiation.

This approach will allow us to propose new clustering of ID/ASD genes integrating their morphological phenotype, their expression pattern during in vitro differentiation and their molecular function, with the aim of proposing new knowledge-based therapeutics.

45.

Molecular basis of autosomal recessive intellectual disability in Pakistani population

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Introduction: Pakistan is a country enriched with genetic resources. Consanguinity adds major part in its genetic enrichment. Cousin marriages are very high (>60%) in Pakistan that cause accumulation of mutations in genetic pool leading to higher ratio of genetic diseases. Intellectual disability (ID) with autosomal recessive mode of inheritance is one of its examples. ID is neurodevelopmental disorder, characterized by significant impairment of cognitive functioning and adaptive behaviors. It has onset before the age of 18 years.

Objective: To identify the underlying genetic defects involved in autosomal recessive intellectual disability (ARID).

Methodology: More than 40 Pakistani families segregating autosomal recessive mode of inheritance were subjected to exome sequencing for mutational analysis. Search for causative mutation was initiated by next generation sequencing that is done by whole-exome sequencing. Results of exome sequencing were further investigated by different bioinformatics-based analysis. A range of DNA variants (n=5-40) remained for each family which is further confirmed by Sanger sequencing.

Results: Some new mutations were found in genes known to be involved in ID. Although our findings are still at preliminary stage, overall data is positive in respect to the objective of our study. Single missense mutation was found in four genes, *TSHR*, *FGFR1*, *SCN4A*, and *AIMP1*. Two missense mutations found in *TMEM67* in two families. Compound heterozygous missense mutations were found in two genes, *SCN1A*, and *DPAGT1*. Non-sense mutation was found in *VPS13B*, and canonical splice site mutation in *MAN2B1*. Two frameshift mutations are found in *APTX*, and *TPO*.

Conclusion: ID is genetically heterogeneous disorder and is very complex in diagnosis. This strategy of whole-exom sequencing seems promising in finding mutations in reported ID genes. Undoubtedly, it would further help in finding possible novel genes involved in ARID.

46.

Genetic elucidation of autosomal recessive intellectual disability

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Identification of the causative genes for autosomal recessive intellectual disability (ARID) has been challenging due to the extreme heterogeneity of the condition and paucity of large consanguineous families. The combination of homozygosity mapping and next generation sequencing (NGS) in consanguineous families has proven to be very helpful in identifying genetic defects in these types of disorders. Here, we have ascertained 100 ID families from the Pakistani population in which there is a high degree of consanguineous marriages (>60%). NGS has been performed on 54 ARID families. Of these, 41 families have been further analyzed by Sanger sequencing. Our data revealed recessive mutations in known ARID genes in 12 families. In another 11 families we identified segregating recessive mutations in a single gene not previously linked to ID, indicating that possible novel genes may have been uncovered. In 12 families multiple segregating rare variants are identified. Four families remained inconclusive, and the results of two families are in progress. A proof-of-concept for the success of our strategy will be presented. In family PKMR14, we have identified a homozygous frameshift mutation in the *ANK3* gene, which segregated with the phenotype in this family. The three affected individuals of PKMR14 showed cognitive deficits, attention/deficit hyperactivity disorder- (ADHD) like phenotype and behavioral problems. Independently, we have identified another inactivating mutation in this gene in a Belgian patient with cognitive deficit, autism, and ADHD. Ankyrin 3 is an adaptor protein that links spectrin cytoskeleton to the integral membrane proteins. It has a crucial role in maintaining the neuronal polarity and molecular organization of the axon initial segments. The causality of *ANK3* mutations in the two families and the role of the gene in cognitive function, were supported by *Drosophila* knockdown model.

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