EUROPEAN SCIENCE FOUNDATION
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EUROCORES Programme
European Collaborative Research

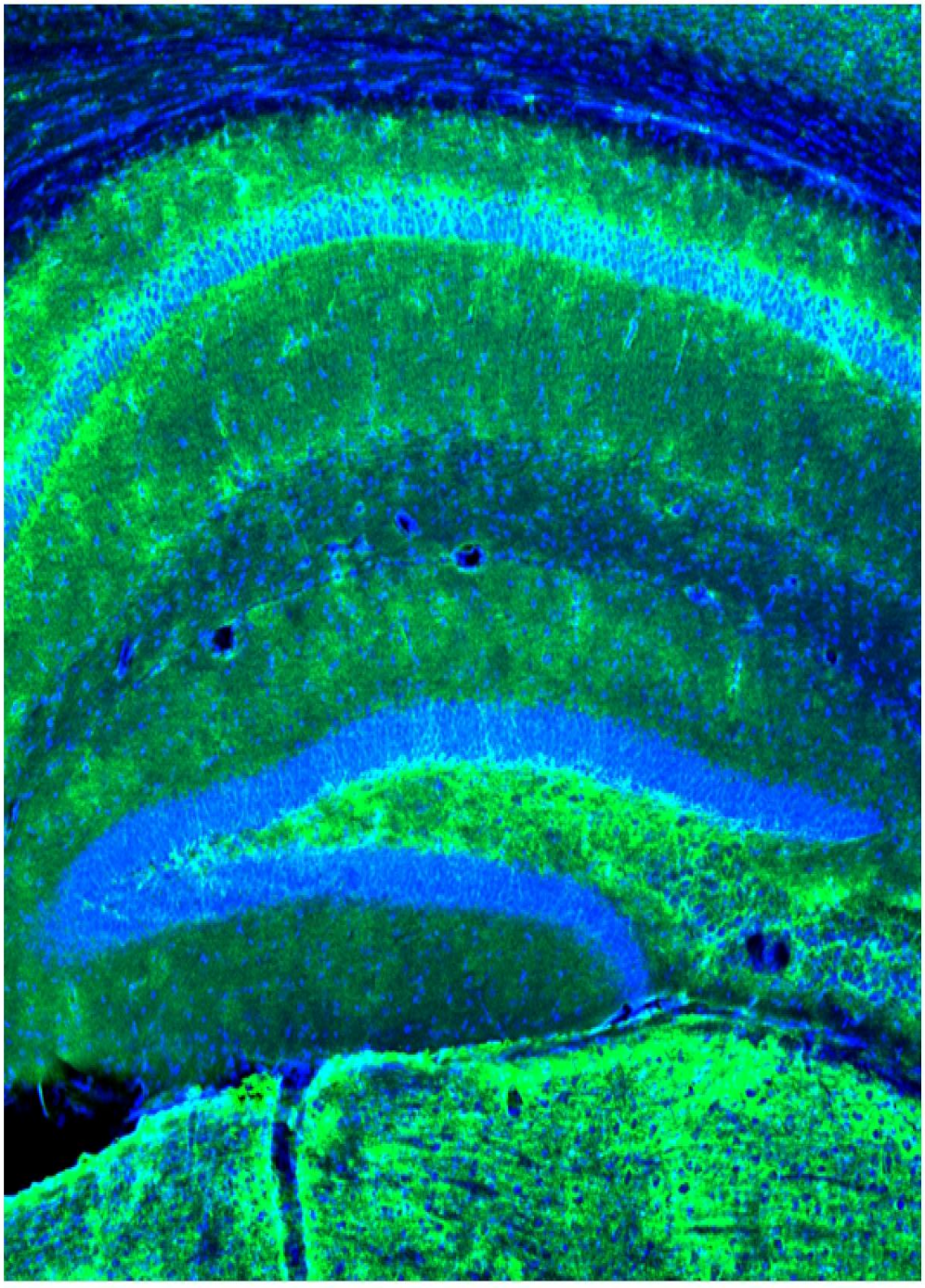
EuroEPINOMICS

Functional Genomic Variation in the Epilepsies

Kick-off Meeting

20 September 2011, Strasbourg, France

Conference Booklet



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Functional Genomic Variation in the Epilepsies

EuroEPINOMICS Kick-off meeting
Strasbourg, 20 September 2011

Programme

Monday 19 September 2011

Location: Restaurant "L'Alsace à Table", 8 rue des Francs-Bourgeois, 67000 Strasbourg

19.30	**CONFERENCE DINNER**

Tuesday 20 September 2011

Location: ESF, Parchemin building, Terra meeting room (ground floor)

9:00	**Welcome and Introduction** Maria Nogueira *(European Science Foundation),* EuroEPINOMICS Coordinator
9:15	**Structure and Objectives of the EuroEPINOMICS Programme** Maria Nogueira
9:35	**Presentation(s) RES** (45 min. + 10 min. discussion) Peter de Jonghe *(University of Antwerp, Belgium),* Project Leader RES
10.30	**COFFEE BREAK**
10:50	**Presentation(s) Epiglia** (20 min. + 10 min. discussion) Erik Taubøll *(Oslo University Hospital, Norway),* Project Leader Epiglia
11:20	**Presentation(s) EpiGENet** (30 min. + 10 min. discussion) Asla Pitkänen *(University of Eastern Finland),* Project Leader EpiGENet
12:00	**Presentation(s) CoGIE** (30 min. + 10 min. discussion) Holger Lerche *(University Hospital Tübingen, Germany),* Project Leader CoGIE

12.40	**LUNCH BREAK**
13.45	**Collaboration with FP7 project** (15 min. + 5 min. discussion) Sanjay Sisodiya *(University College London, UK),* Associated Partner EpiGENet
14.05	**EPICURE** (15 min. + 5 min. discussion) Giuliano Avanzini *(Fondazione IRRCS, Milan, Italy),* Invited Speaker

Scientific Committee meeting (mandatory for Project Leaders but open to all participants):

14.30	Networking Activities
15.15	**COFFEE BREAK**
15.35	Dissemination Activities
16.00	Reporting Procedures
16.15	Other programmes / initiatives related to EuroEPINOMICS science
16.25	Any other business
16.30	Closure of meeting

Giuliano Avanzini

Fondazione IRRCS
Istituto Nazionale
Neurologico Carlo Besta
via Celoria 11
20133 Milan
Italy

Invited Speaker

EpiCure

The EU collaborative integrated project EPICURE had been developed involving several groups from14 European countries to address several important questions in epilepsy research:

Epilepsy genes and functional alterations affecting the encoded proteins. The genome wide association study identified recurrent microdeletions that are significantly associated with IGE and play a substantial epileptogenic role in up to 5% of IGE patients: 15q13.3 microdeletions constitute the most prevalent risk factor for common epilepsies identified to date.

Voltage-gated and ligand-gated ion channels dysfunctions due to mutations of the coding genes or to exogenous factors was intensively investigated using complementary advanced methods

Six Nav1 mutations resulting in a folding defective channel that can be rescued by interactions with associated proteins and drugs have been identified. A rescuable folding defect seems to be a common pathogenic mechanism for Nav1.1 epileptogenic mutants and may account for phenotype heterogeneity.

Marked changes in GABAA- receptor subunit expression were characteried in temporal lobe epilepsy. The loss of phasic GABAergic inhibition increases the gain of the neuron, whilst increased tonic inhibition changes the offset making the neuron less excitable. The effect in the epileptogenic network is complex

We found that inflammation is an important epileptogenic factor and was found to lead to a strong reduction in Ih current, indicating that inflammation can by itself trigger a channelopathy.

Developmental aspects of epileptogenesis By mRNA expression of layer-specific genes cortical and periventricular heterotopia were shown to have a pseudo-laminar structure. The distribution of GABAergic cells was altered since the embryonic stages, as a consequence of the derangement of tangential fibres. The membrane proteins aquaporin and connexion are differentially overexpressed in human dysplastic tissue and may have a significant role in epileptogenesis. Five new genes responsible for malformation of cortical development have been identified and animal models have been developed. A three-tiered Dysplasia Classification system have been developed Epilepsia [52 (2011);158–1) as an important deliverable that EPICURE offers to the international epilepsy community.

New antiepileptic strategies and pharmacoresistance

Five genes have been identified that could provide valuable clues to unravelling the mechanisms of antiepileptic drug resistance and to predicting response to pharmacological therapy.

The potential of innovative antiepileptic strategies based on gene, stem cell therapy, vehicles capable of transporting effective agents into the epileptogenic zone (mesoangioblasts, viral vectors, encapsulated cells) have been investigated.

Besides the scientific results the EPICURE project ends up with some “products” that will widely benefit the scientific community such as innovative methods and techniques that had been developed during the project and a general tissue brain bank that has been established in Erlangen.

I believe that EPICURE substantially contributed in establishing a network of excellence in epilepsy research and created a momentum which will have an important impact in fostering future development of epilepsy research in Europe. We are glad and proud for being involved in it.

Peter De Jonghe

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RES

Unraveling Rare Epilepsy Syndromes: a clinical and molecular genetics approach

The RES CRP aims to decipher the genetic basis of many RES by bringing together the expertise of epileptologists with access to large patient cohorts and molecular genetic teams with a vast experience in locus and gene identification.

Collectively, this team of researchers will recruit the largest cohort of patients with RES to date and, for the first time, collect comprehensive clinical, electrophysiological and genealogical data in a standardized way. Novel genes for seizure disorders will be identified using broad range of technologies including large-scale CNV analysis and next-generation sequencing techniques. These technologies will be applied in a systematic genetic workflow to streamline analysis efficiency. Finally, genotype-phenotype correlation will be performed to identify novel disease entities based on genetic findings.

Holger Lerche

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Hertie Institut für Klinische Hirnforschung
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Germany

CoGIE

Complex genetics of idiopathic generalized and rolandic epilepsy

Epilepsy is a common neurological disorder affecting 0.5-1% of the population. Idiopathic generalized epilepsy (IGE) and Rolandic epilepsy (RE), the most common idiopathic syndromes, are largely genetic and represent prototypes for common diseases with complex inheritance. Rare mutations (mainly in ion channels) have been identified in monogenic IGE or as risk factors in IGE and RE. In approximately 3% of IGE patients, microdeletions have been identified but the vast majority of genetic risk factors predisposing to both diseases remains to be identified.

The objective of the 'Complex Genetics of Idiopathic Epilepsies' consortium (CoGIE) is to unravel the genetic basis and pathophysiology of IGE and RE. The consortium aims to identify both common and rare disease-relevant genetic variations on a genome-wide basis and to determine the functional role of identified variants. CoGIE exploits an established and unique interdisciplinary research network of clinicians, geneticists, biostatisticians, physiologists and neuroanatomists.

CoGIE will expand the largest cohorts of well-characterized IGE and RE patients already collected by CoGIE partners and other collaborators within the European EPICURE project. By using a combination of modern genetic techniques, including next generation sequencing, genome-wide association studies, copy number variation analysis, and high-throughput SNP genotyping, we will identify and validate the genetic risk factors for IGE and RE. Subsequently, comprehensive biostatistical analysis of the genetic data generated will be applied to explore the common etiological factors underlying IGE and RE. Selected genetic variants will be characterized functionally using automated and conventional electrophysiological tech-niques and expression in neurons, and neuroanatomical studies will determine the neuronal expression patterns of affected genes. We anticipate that our studies will have the power and novelty to reveal new pathophysiological pathways of common idiopathic epilepsy syndromes.

Asla Pitkänen

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EpiGENet

Epigenetic Pathomechanisms Promoting Epileptogenesis in Focal and Generalized Epilepsies

About 1% of the population has epilepsy, and approx. 30% of the patients lack response to currently available antiepileptic drug treatment. Onset and progression of spontaneous drug-resistant seizure activity remains, however, difficult to predict and determine in affected patients, irrespective of their epileptogenic condition, i.e., traumatic brain injury, temporal lobe (hippocampal) sclerosis or genetic inheritance. The objective of this CRP is to characterize common epigenetic pathomechanisms of epileptogenesis and, thereby, to identify novel targets for pharmacotherapy. Experimental animal models and well-characterized samples from epilepsy patients will be available for genome wide association studies, massive parallel sequencing of the methylome and gene expression analysis. Bioinformatic integration of these valuable data pools will help to elucidate candidate epileptogenesis genes (CEG) and common traits for altered CEG expression.

Our CRP comprises expertise in different methodological approaches, i.e. genetic platform technologies, functional and molecular studies in animal models and human samples. Highly visible interaction and collaboration will be organized between our 6 IPs and 3 APs to exchange data and sample pools. In addition, specialized expertise will be made available to the consortium to validate aberrant epigenetic regulation patterns in common forms of epilepsies. These results will lead us to translational studies addressing preclinical trials for epigenetic drug treatment or shRNA interference. Continuous video-EEG monitoring will clarify the impact of either strategy to medically attenuate or prevent the progression of chronic epilepsy in clinically relevant animal models. This CRP consists of 9 laboratories from 6 European countries and Australia with a long-standing track record in genetic and functional research in both epilepsy models and human samples. It also promotes the European Epilepsy Brain Bank, which provides standardized human brain specimens for scientific research across European countries. The novelty of our CRP objectives and published expertise of all applicants will generate definitive results.

Sanjay Sisodiya

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Institute of Neurology
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United Kingdom

Invited Speaker

Collaboration with FP7 project 'EpiPGX'

EpiPGX is an EU Framework Programme 7 funded project examining the pharmacogenomics of antiepileptic drugs. It is a multicentre project, building upon several existing and ongoing collections of phenotype and genotype information from people with epilepsy. New cases will also be recruited. A variety of methods, including GWAS and high-throughput sequencing, will be used to evaluate the contribution of genetic variation to carefully-defined response phenotypes. We welcome collaboration. This presentation will outline the project and seek to establish collaboration with EuroEPINOMICS projects.

Erik Taubøll

Oslo University Hospital - Rikshospitalet
Faculty of Medicine
Department of NeurologySognsvannsveien 20
0027 Oslo
Norway

Epiglia

EpiGlia

Temporal lobe epilepsy (TLE) consists of several subgroups of which Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS) is the most severe one. There is also a high association between TLE and febrile seizures. To identify the different TLE subgroups may open new possibilities to tailor pharmacological treatment. Recent findings suggest that modified glial function may play an important role in the hyperexcitability of neuronal tissue promoting epileptogenesis and disease progression, specifically in TLE.

This project tests the hypothesis that astrocytes play a critical role in generation, spreading, and maintenance of seizures in different TLE subgroups. To do this we aim to focus on genetic studies on glia targets, functional studies in living human epileptic tissue and MTLE-HS mouse models including febrile seizures, studies on knockout animals, and other functional and molecular biological studies.

Albert Becker

University of Bonn
Neuropathology
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Germany

Project
EpiGENet

Analysis of the Cav3.2 promoter and interference with its activation – implications for epileptogenesis.

Karen M.J. van Loo, Katharina Pernhorst, Susanne Schoch, Albert J. Becker
Dept. of Neuropathology, University of Bonn Medical Center

Temporal lobe epilepsy (TLE) is one of the more common seizure disorders in adults. The underlying mechanisms during its etiopathogenesis, collectively referred to as epileptogenesis, are however poorly understood. Recently, we have identified the T-type Ca2+ channel Cav3.2 as a pivotal player in the pilocarpine model of epileptogenesis. Here, we aim to identify the molecular signaling cascades involved in Cav3.2 transcriptional regulation. Bioinformatic analysis of the predicted Cav3.2 promoter revealed binding sites for a number of transcription factors (TFs), including epileptogenesis-associated early growth response 1 (Egr1), cAMP responsive element binding protein 1 (CREB1), metal-regulatory transcription factor 1 (MTF1) and nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFkB1). Interestingly, overexpression of Egr1, CREB1 and MTF1 in neuronal NG108-15 cells resulted in Cav3.2 promoter activation, whereas a dominant-negative variant of NFkB1 could counteract Cav3.2 upregulation. Together, our findings suggest that alterations in Egr1, CREB1, MTF1 and NFkB1 might regulate Cav3.2 expression levels and, consequently, provide new possibilities for pharmacological intervention aimed at preventing the process of epileptogenesis.

S. Hande Caglayan

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Project
RES

Genome-wide linkage scan for new susceptibility loci in a large kindred with GEFS+ phenotype and phenotyping patients with monogenic forms of epilepsy and Epileptic Encephalopathies

S. Hande Caglayan
Department of Molecular Biology and Genetics, Bogazici University

GEFS+, a distinct autosomal dominant disorder with 50-60% penetrance is characterized by febrile seizures beyond the age of 6 and with the presentation of different generalized or focal seizures. The most relevant gene to the phenotype is SCN1A and mutations in SCN1B, SCN2A, GABRG2 and GABRD were also identified in a few families. All mutations in these genes are inherited as autosomal dominant traits; so far no genes for autosomal recessive GEFS+ have been identified. About 2,000 inhabitants of a village located in the Aegean Region of Turkey are descendants of 5 core unrelated families settled in the area in the early 20th Century forming a multigenerational family with multiple consanguineous marriages with approximately 40% of the individuals exhibiting a GEFS+ phenotype and other clinical features, such as migraine and depression.

Initial DNA sequence analysis of the coding regions of SCN1A, SCN1B, SCN2A, and GABRG2 genes in the index patient did not reveal any pathogenic changes, suggesting that a novel genetic defect underlies

	the GEFS+ phenotype in this family. The objective of this proposal is to identify the novel GEFS+ locus and gene (or eventually several novel loci) in two large family branches by linkage and homozygosity mapping in selected individuals affected by GEFS+ and other associated features (Aim 1). Genome-wide linkage analysis using high-density SNP arrays will be performed using both parametric and non-parametric models as well as homozygosity mapping to identify disease-associated chromosomal regions. Subsequently, the causal mutation will be identified by sequence analysis of positional candidate genes and/or locus sequencing using next-generation sequencing technologies. In case the culprit gene turns out to be an ion channel, functional in vitro studies will be initiated by AP3 of this consortium. In addition to this specific task, we will make several contributions to the common goals of this CRP by sampling and phenotyping patients with monogenic forms of epilepsy and Epileptic Encephalopathies (Aim 2). These phenotypic data and DNA samples will be made available to other IPs who perform mutation detection and CNV analysis. In the domain of the monogenic forms we can make unique contributions by sampling consanguineous families with autosomal recessive epilepsies from the Turkish population and genetic isolates. To this end, the CRP-RES initiative will be widely announced among clinicians through the Turkish League Against Epilepsy (TLAE) Genetics Commission.
Katja Kobow University Hospital Erlangen Institute of Neuropathology Schwabachanalge 6 91054 Erlangen Germany **Project** EpiGENet	**Altered DNA promoter methylation in experimental TLE** Katja Kobow, Ina Fritzsche, Angelika Mühlebner, Marleisje Njunting, Jan Hauke, Eric Hahnen, Roland Coras, Ingmar Blümcke *Institute of Neuopathology, University Hospital Erlangen (KK, IF, MN, RC, IB); Institute of Human Genetics, University of Cologne (JH, EH); Department of Pediatrics, Medical University Vienna (AM)* Aberrant epigenetic chromatin modifications have been increasingly recognized in many neurological disorders including autism, bipolar disorders, schizophrenia, brain tumors and neurodegeneration. These epigenetic lesions include changes in localized or global density of DNA methylation, and aberrant histone modification (acetylation, methylation, phosphorylation, ubiquitination, sumoylation), and are likely to be acquired by environmental or intrinsic factors thereby promoting the pathogenic condition. Herein, we propose aberrant promoter methylation as important pathomechanism underlying epileptogenesis. Indeed, we were first showing increased levels of DNA promoter methylation in human temporal lobe epilepsy (Kobow et al., 2009). However, the pathogenic impact of epigenetic DNA modification on neuronal excitability and epileptogenesis awaits experimental confirmation. Therefore, we quantitatively examined promoter methylation and gene expression of the candidate epileptogenesis gene mGluR2 (metabotropic glutamate receptor 2) in the rat pilocarpine model, as well as consequences on epigenetic DNA modification using ketogenic diet (KD) treatment. We identified time- and region-specific down-regulation of mGluR2 in pilocarpine-treated rat HC compared to cerebellum and untreated control samples. Concomitantly, hippocampal mGluR2 promoter DNA methylation as well as gene expression of de novo DNA methyltransferase 3b (Dnmt3b) was increased. This epigenetic mechanism could be

	repressed by administration of a high-fat, high-protein, low carbohydrate ketogenic diet (KD), which also reduced seizure frequency in our animal model. Preliminary data from methylation specific immunofluorescence analysis suggested that DNA methylation is globally affected in brain regions targeted by chronic seizure activity and may be an important pathogenic trigger for epileptogenesis. Antiepileptic KD treatment completely antagonized DNA methylation and showed a distinct correlation between seizure frequency and DNA promoter methylation pattern in our animal model. Studying epigenetic chromatin modifications will open fascinating avenues for our understanding of common pathomechanism in epileptogenesis. In addition, novel epigenetically active pharmacological compounds may be recognized as AED treatment and rapidly translate into new clinical perspectives.
Johannes Lemke University of Bern Children's Hospital Human Genetics Inselspital 3010 Bern Switzerland **Project** RES	**Validating massive parallel sequencing as a diagnostic tool for seizure disorders** J. Lemke 1, E. Riesch 2, J. Hansen 1, T. Scheurenbrand 2, C. Courage 1, S. Gallati 1, T. Dorn 3, M. Wolff 4, Y. Weber 5, H. Lerche 5, D. Boehm 2, S. Biskup 2 *1 Department of Paediatrics, University Hospital Inselspital, Bern, Switzerland, 2 CeGaT GmbH, Tuebingen, Germany, 3 Swiss Epilepsy Center, Zurich, Switzerland, 4 Department of Paediatrics, University of Tuebingen, Tuebingen, Germany, 5 Department of Neurology, University of Tuebingen, Tuebingen, Germany* Introduction: The epilepsies are common neurological disorders with a strong genetic impact. Consequently, understanding the genetic basis of seizure disorders will provide novel insights into the underlying pathophysiology and result in novel diagnostic and therapeutic avenues. With our approach we aim to reveal the genetic basis of epileptic disorders in so far unresolved cases. Methods: Genomic DNA is enriched for a panel of 485 genes using a custom designed Agilent SureSelect in solution kit. 265 of the 485 genes are known causative genes for seizure disorders comprising all relevant epilepsy phenotypes. The remaining genes on the panel represent putative candidate genes for epileptic disorders. Sequencing is performed on the SOLiD 4 platform. We developed a diagnostic pipeline to identify potentially pathogenic SNVs, small insertions and deletions as well as larger indels including whole exon deletions and duplications. Variants and aberrations are validated using conventional methods. Results: We sequenced more than 20 so far genetically undiagnosed familial and isolated cases with a broad spectrum of epilepsy phenotypes. We present an overview of the number of detected sequence alterations comprising mutations, possibly damaging variants as well as benign SNPs in both well-known epilepsy genes and putative candidates. Aberrations that so far have been detected and confirmed in our cohort comprise mutations in frequently or occasionally affected genes (e.g. SCN1A, SCN2A, STXBP1) as well as uncommonly or rarely mutated genes (e.g. KCNJ10, CACNA1H). Conclusion: We have successfully established a fast and cost efficient genetic screening method for patients with seizure disorders. By applying this approach we hope to uncover both known and unknown

	sequence variants and give new insights in genetic factors involved in epileptogenesis.
Snezana Maljevic Tübingen University Hertie Institute for Clinical Brain Research Neurology and Epileptology Otfried-Müller-Str. 27 72076 Tübingen Germany **Project** CoGIE	**Pathophysiological mechanisms of idiopathic epilepsies** Snezana Maljevic, Holger Lerche *Dept. of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University Clinic Tübingen, Tübingen, Germany* Idiopathic epilepsies have been associated with mutations affecting different ion channel genes. Our lab has been performing diverse functional studies of ion channel variants associated with these forms of epilepsy. We combine several approaches, including (i) functional examination of the detected ion channel mutations using conventional and automated patch- and two-voltage clamp techniques in heterologous expression systems, (ii) their expression in primary neuronal cultures and (iii) analysis of network activities in mouse models carrying mutations or lacking genes associated with idiopathic epilepsies. We are further interested in specific effects of a disturbed chloride homeostasis in different parts of the brain on epileptogenesis and have been analyzing developmental expression patterns and physiological roles of affected ion channels, especially those localized at the axon initial segment. Studying the effects of epilepsy mutations in heterologous systems and neurons should increase our understanding of the regulation of brain excitability and provide insights into the pathophysiological mechanisms underlying idiopathic forms of epilepsy, pacing the way for new therapeutical approaches.
Katja Menzler University Hospital Giessen and Marburg Epilepsiezentrum Hessen (EZH) Baldingerstr 35033 Marburg Germany **Project** RES	**Microstructural imaging in patients and healthy subjects with genetic variants of the SCN1A gene relevant for epilepsies and pharmacoresistance** Menzler, K.; Hermsen, A.; Belke, M.; Reif, P. S.; Klein, K. M.; Haag, A.; Knake, S. & Rosenow, F. *Epilepsy Center Marburg, Philipps-University Marburg, Germany* Background: Mutations in the SCN1A gene are related to several types of monogenic epilepsy syndromes. Additionally, the polymorphism SCN1A-SNP rs3812718 is associated with differences in cortical excitability as measured by transcranial magnetic stimulation (TMS) and with the dosage of several antiepileptic drugs. The aim of this study is to evaluate microstructural brain changes in healthy subjects with different genotypes of the polymorphism SCN1A-SNP rs3812718 and in family members of families with rare epilepsy syndromes (RES) related to sodium channel mutations and to evaluate a possible correlation with changes in cortical excitability. Methods: 120 healthy subjects (40 each with genotype AA, AG and GG of the polymorphism SCN1A-SNP rs3812718) and 60 family members of families with RES related to sodium channel mutations will be included in the study. Microstructural brain changes will be investigated using Diffusion Tensor Imaging (DTI). Cortical excitability will be evaluated using TMS (parameters: resting motor threshold [RMT], short intracortical facilitation [SICF], short intracortical

	inhibition [SICI] and cortical silent period [CSP]). Current state: The majority of the healthy subjects have received the TMS investigation and the first few subjects have been investigated by DTI. The family members of families with RES need to be identified and recruited.
Bernd Neubauer University of Giessen Facutly of Medicine and Pediatrics Department of Pediatric Neurology Feulgenstr. 12 35440 Giessen Germany **Project** CoGIE	**Genetic variation in Rolandic Epilepsy** B. Neubauer, P. Nürnberg, D. Lal, T. Sander *Department of Neuropediatrics Giessen (BN) and Cologne Genome Center (P.N., D.L., T.S.)* The goal of this project is to unravel the genetic basis of benign epilepsy of childhood with centrotemporal spikes i.e. Rolandic epilepsy (RE), the most common focal idiopathic epilepsy syndrome, accounting for 15 % of all childhood epilepsies. The syndrome is defined by: Brief, simple, partial, hemifacial motor seizures, frequently having associated somatosensory symptoms. These partial seizures have a tendency to evolve into generalized tonic clonic seizures. The electroencephalographic hallmark of RE, a prerequisite for diagnosis, are blunt high-voltage characteristically shaped centrotemporal spikes (CTS). The genetic etiology of RE is unequivocal and it is generally accepted that, like in all other common idiopathic epilepsies, a complex mode of inheritance involving multiple genes and environmental influences appears most likely. It has long been reported that in families with RE, not the epilepsy, but the associated EEG trait follows an autosomal dominant mode of inheritance with high but incomplete penetrance and age dependency (e.g. Heijbel et al. 1975). Very recently, independent family studies corroborated these findings (Bali et al. 2007). We reported linkage to markers on 15q13.3 (D15S165 and D15S1010) in 22 families with RE (Neubauer et al. 1998). Later, this locus was found to harbor a recurrent microdeletion encompassing the CHRNA7 gene responsible for 1% of cases with idiopathic generalized epilepsy (IGE) (Helbig et al. 2009). In our set of 90 RE families, only one family revealed this microdeletion (unpublished). Recently, we identified variations in KCNQ2 and KCNQ3 as susceptibility factors in some families with RE. Notably, the revealed sequence variations were also detectable in a large sample of IGE patients ($p = 0.008$) (Neubauer et al. 2008). In the mean time we have ascertained a sample of 105 families with sibpairs affected by RE (plan = 125) and 53 parent-offspring trios (plan = 375) with RE (collaboration with IP4, F. Zimprich). IP3 aims to identify susceptibility genes for RE by applying genome-wide linkage and association analyses (collaboration with IP1, P. Nürnberg and AP4 T. Sander), copy number variation (CNV) studies, and next-generation sequencing (NGS) (collaboration with IP1, P. Nürnberg, and IP4, F. Zimprich).
Stephanie Schorge UCL Institute of Neurology University College London Institute of Neurology Department of Clinical & Experimental Epilepsy	**Drugs, mutations and splicing converge to modify the stability of inactivation of type 1 sodium channels encoded by SCN1A** Emily V Fletcher, Klaus Wanisch ,Dimitri M Kullmann & Stephanie Schorge

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Project
EpiGENet

UCL Institute of Neurology, London WC1N 3BG, UK

Although highly conserved, the functional role of alternative splicing in sodium channels remains unclear. In SCN1A, a polymorphism that disrupts this splicing has been associated with altered response to anti-epileptic drugs (AEDs) and potentially with an increased likelihood of developing febrile seizures. We asked if this splicing had an impact on the intrinsic properties of NaV1.1 channels that is consistent with a role for alternative splicing in the development or treatment of seizures. We compared the behaviour of two splice variants (NaV1.1-5A and NaV1.1-5N) with and without the R1648H mutation, which is associated with monogentic epilepsy in a large kindred, and also in the presence of a frontline AED, phenytoin, which works primarily through stabilising the inactive state of neuronal sodium channels. At physiological temperatures the rate at which the splice variants recovered from inactivation, a characteristic strongly associated with AED efficacy, was faster for NaV1.1-5N than NaV1.1-5N channels. This difference was accounted for by a single amino acid substitution, aspartate to asparagine, in the S3-S4 linker of the first domain, and was occluded by the R1648H mutation and also by phenytoin. Alternative splicing, phenytoin and a mutation that causes epilepsy thus converge to modulate the rate of recovery of currents mediated by NaV1.1, underlining the importance of this parameter in human seizure susceptibility and treatment. We are interested in whether a polymorphism known to disrupt inclusion of exon 5N is associated with altered severity of epilepsies associated with loss of SCN1A function.

Fritz Zimprich

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Austria

Project
CoGIE

Multiple rare variants in Rolandic epilepsy

Fritz Zimprich, Eva Maria Reinthaler
Dept. of Clinical Neurology, Medical University of Vienna

The main aim of the IP4-Project of the CoGIE-CRP will be to identify rare causative variants for Rolandic epilepsy. We plan to perform whole exome sequencing in patients with Rolandic epilepsies (enriched for familial cases) (As many cases as possible depending on the sequencing costs). We will then define candidate genes for Rolandic epilepsy based on the results of the exome sequencing stage and after considering results from other IPs on idiopathic generalized epilepsies. Candidate genes will be re-sequenced in further samples of Rolandic epilepsy patients to confirm any detected mutations.

List of Participants

Surname	Firstname	Town	Country	CRP	Status
Albers	Johanna	Kiel	DE	RES	Project Member
Avanzini	Giuliano	Milan	IT	not applicable	Invited Speaker
Balling	Rudi	Luxembourg	LU	CoGIE	Principal Investigator
Barisic	Nina	Zagreb	HR	RES	Associated Partner
Becker	Albert	Bonn	DE	EpiGENet	Principal Investigator
Blümcke	Ingmar	Erlangen	DE	EpiGENet	Principal Investigator
Brilstra	Eva	Utrecht	NL	CoGIE	Project Member
Caglayan	S. Hande	Istanbul	TR	RES	Principal Investigator
Craiu	Dana	Bucharest	RO	RES	Principal Investigator
de Graan	Pierre N.E.	Utrecht	NL	RES	Associated Partner
De Jonghe	Peter	Wilrijk	BE	RES	Project Leader
del Sol	Antonio	Luxembourg	LU	RES	Project Member
Helbig	Ingo	Kiel	DE	RES	Principal Investigator
Heuser	Kjell	Oslo	NO	Epiglia	Project Member
Hoffman-Zacharska	Dorota	Warsaw	PL	RES	Principal Investigator
Huusko	Noora	Kuopio	FI	EpiGENet	Project Member
Kälviäinen	Reetta	Kuopio	FI	Epiglia	Principal Investigator
Kobow	Katja	Erlangen	DE	EpiGENet	Project Member
Lehesjoki	Anna-Elina	Helsinki	FI	CoGIE	Principal Investigator
Lemke	Johannes	Bern	CH	RES	Principal Investigator
Lerche	Holger	Tübingen	DE	CoGIE	Project Leader
Lukasiuk	Katarzyna	Warsaw	PL	EpiGENet	Principal Investigator
Maljevic	Snezana	Tübingen	DE	CoGIE	Project Member
Menzler	Katja	Marburg	DE	RES	Project Member
Neubauer	Bernd	Giessen	DE	CoGIE	Principal Investigator
Nogueira	Maria Manuela	Strasbourg	FR	not applicable	ESF staff
Nürnberg	Peter	Köln	DE	CoGIE	Principal Investigator
Pitkänen	Asla	Kuopio	FI	EpiGENet	Project Leader
Reinthaler	Eva	Vienna	AT	CoGIE	Project Member
Rosenow	Felix	Marburg	DE	RES	Principal Investigator
Sander	Thomas	Cologne	DE	EpiGENet	Principal Investigator
Schneider	Reinhard	Luxembourg	LU	CoGIE	Project Member
Schorge	Stephanie	London	UK	EpiGENet	Associated Partner
Selmer	Kaja	Oslo	NO	RES	Principal Investigator
Serratosa	Jose M	Madrid	ES	RES	Principal Investigator
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