epiXchange 2018
23 May 2018, Brussels

A community building event for epilepsy research

Programme & Abstracts
Dear Colleagues and Guests,

It is our pleasure to welcome you to the epiXchange conference here at THE EGG in Brussels.

This conference is the first milestone of more than a year’s collaboration between seven FP7 epilepsy research projects to define priorities in future epilepsy research and create synergies between projects. The outcomes of the conference will be published in a Position Paper highlighting the priorities for epilepsy research.

The symposium is a great opportunity to learn about the recent achievements and their impact in the field of epilepsy; it allows you to network with renowned experts and get acquainted with the activities of other on-going and future initiatives.

Presentations from epiXchange partners will showcase the project success stories; a poster session will then allow for deeper discussions. You will learn how the extensive research efforts during the last 5 years can be translated into solutions for diagnosing and treating epilepsy.

Sincerely,
The epiXchange Project Coordinators

Renzo Guerrini (DESIRE)  Sergiusz Jóżwiak (EPISTOP)
Helen Cross (EpiCARE)  Mérab Kokaia (EPITARGET)
David Henshall (EpimiRNA)  Michele Simonato (Epixchange)
Sanjay Sisodiya (EpiPDX)

The epiXchange Project Coordinators

The EU Journey in Epilepsy Research

This conference marks a key milestone of ‘The EU Journey in Epilepsy Research’. The journey started with the European Parliament’s ‘Written Declaration on Epilepsy’ back in 2011. The European Parliament statement supported the priority of the European Commission to invest EUR 42 million in 2013 to investigate the pathophysiology, prevention, patient stratification and identification of new drug targets in epilepsy.

This conference will show the amazing results stemming from the projects covering these areas, some of which have already been showcased in our Horizon 2020 Magazine. But the story does not stop there. This cluster of projects, alongside other projects funded from the EU Research Health Programme and the Marie Curie Actions, has created a community of researchers that has shaped the European Reference Network on rare and complex epilepsies, which is supported by the Directorate-General Health and Food Safety’s Health Programme. All these efforts truly maximise the impact of EU funded research for the benefit of patients. This journey exemplifies that through European collective willpower, we do make a big difference in Europe and globally.

Another chapter of ‘The EU Journey in Epilepsy Research’ now begins with this conference.

Line Matthiessen
Acting Director for Health
DG Research & Innovation
European Commission
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Programme</td>
<td>6</td>
</tr>
<tr>
<td>General Overview Venue</td>
<td>10</td>
</tr>
<tr>
<td>Chairs &amp; Speakers, Biographies &amp; Abstracts</td>
<td>13</td>
</tr>
<tr>
<td>Translational Research in Biomarkers</td>
<td>15</td>
</tr>
<tr>
<td>Genetics of Epilepsy – Therapeutics Implications</td>
<td>29</td>
</tr>
<tr>
<td>Innovation Therapeutics &amp; Translation</td>
<td>41</td>
</tr>
<tr>
<td>Understanding Co-Morbidities in the Epilepsies</td>
<td>59</td>
</tr>
<tr>
<td>Biobanks and Databases – Basis for Translational Research</td>
<td>73</td>
</tr>
<tr>
<td>Poster Abstracts</td>
<td>87</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>89</td>
</tr>
<tr>
<td>Genetics</td>
<td>101</td>
</tr>
<tr>
<td>Therapeutics</td>
<td>111</td>
</tr>
<tr>
<td>Co-Morbidities</td>
<td>129</td>
</tr>
<tr>
<td>Biobanks and Databases</td>
<td>133</td>
</tr>
<tr>
<td>Imprint</td>
<td>136</td>
</tr>
<tr>
<td>Time</td>
<td>Topic</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>08:00</td>
<td>Welcome and Registration</td>
</tr>
<tr>
<td>08:30</td>
<td>Welcome Speeches: Overview of epiXchange Projects</td>
</tr>
<tr>
<td>09:00</td>
<td>A Decade of EU Efforts on Epilepsy Research</td>
</tr>
<tr>
<td></td>
<td>A Decade of EU Efforts on Epilepsy Research</td>
</tr>
<tr>
<td></td>
<td>A Decade of EU Efforts on Epilepsy Research</td>
</tr>
<tr>
<td>10:30</td>
<td>Coffee Break and Poster Session</td>
</tr>
<tr>
<td>11:00</td>
<td>Genetics of Epilepsy - Therapeutics Implications</td>
</tr>
<tr>
<td></td>
<td>Genetics of epileptogenic disorders: update and consequences on patients' therapeutic management</td>
</tr>
<tr>
<td></td>
<td>The emerging role of genetics in the clinical care pathway</td>
</tr>
<tr>
<td></td>
<td>Genetic variation in microRNAs and their targets in epilepsy</td>
</tr>
<tr>
<td></td>
<td>Predictors of responses to AEDs</td>
</tr>
<tr>
<td></td>
<td>Genetics and mosaicism in Tuberous Sclerosis Complex: preliminary analysis of association with seizure phenotype in EPITOP</td>
</tr>
<tr>
<td></td>
<td>Functional epigenomic dissection of epilepsy in human brain tissue and corresponding mouse models</td>
</tr>
<tr>
<td>12:15</td>
<td>Lunch and Poster Session</td>
</tr>
<tr>
<td>13:35</td>
<td>Innovation Therapeutics &amp; Translation</td>
</tr>
<tr>
<td></td>
<td>Short overview of current status of research, impact and gaps to be filled in the area of therapeutics</td>
</tr>
<tr>
<td></td>
<td>Innovative therapeutic strategies for epileptic encephalopathies</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Improving clinical trials in epilepsy: addressing the hurdles and filling the gaps

MicroRNAs as targets for seizure control and disease-modification

Presense vs postseizure treatment of epilepsy - pros and cons

Evaluation of tolerability and antiepileptogenic efficacy of multitargeted drug combinations by a two-stage approach

Differential effect of neuropeptides on excitatory synaptic transmission in human epileptic hippocampus

15:15

Understanding Co-Morbidities in the Epilepsies

Short overview of the current status of research, impact and gaps to be filled in the area of co-morbidities

Perspectives for investigating physiopathological mechanisms of co-morbidities in epilepsy

Predictors of response and resistance and co-morbidities

Autism in tuberous sclerosis early developmental trajectories, potential biomarkers and treatment strategies

Systems genetic evidence for a convergence of epilepsy and its co-morbidities on shared molecular pathways

Effects on seizures and epilepsy co-morbidities of encapsulated BDNF-producing cells

16:25

Coffee Break and Posters

16:55

Biobanks and Databases - Basis for Translational Research

Short overview of the current status of research, impact and gaps to be filled in the area of biobanks and databases

Success of the European Epilepsy Brain Bank

Multi-omic atlas of functional microRNA for epilepsy

Controlled access resources for genetic and pharmacogenetics research for epilepsy

Biobanking in Tuberous Sclerosis Complex: challenges and opportunities for understanding epilepsy and cognitive and behavioural co-morbidities

Database for powered preclinical multicenter trial

18:05

Closing Remarks

18:15

End of Conference
General Overview Venue

THE EGG is a site spread out over 5000m² in the heart of Brussels. It is a unique modular space housed in a post-industrial building, offering easy access and many different parking facilities in the vicinity.
Chairs & Speakers
Biographies & Abstracts

1 Translational Research in Biomarkers
2 Genetics of Epilepsy - Therapeutics Implications
3 Innovation Therapeutics & Translation
4 Understanding Co-Morbidities in the Epilepsies
5 Biobanks and Databases - Basis for Translational Research
Translational Research in Biomarkers

Chairs & Speakers
Biographies & Abstracts
Asla Pitkänen
Chair Biomarkers

Biography
Asla Pitkänen is Professor of Neurobiology at the University of Eastern Finland. Her neuroscience research at the A. I. Virtanen Institute ranges from the cellular and molecular level to experimental animal models and brain imaging. Her research goal is to identify early pathophysiological mechanisms following traumatic brain injury, which can be used as biomarkers and treatment targets to prevent post-traumatic epileptogenesis. She is involved as a Principal Investigator and Workpackage Leader in the EU-funded research project EPITARGET (www.epitarget.eu) amongst others.

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University of Eastern Finland

Project
EPITARGET

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Michele Simonato
Chair Biomarkers

Biography
Michele Simonato, MD, is Professor of Pharmacology and Toxicology at the Universities of Ferrara and San Raffaele (Milan), Italy. He carried out his training in Italy and in the USA. He is Chair of a joint task force of the International League against Epilepsy and the American Epilepsy Society aiming to optimise development of new anti-epilepsy therapies. He is Vice President of the Italian National Institute of Neuroscience. His research focuses on the development of new therapeutic approaches for epilepsy, in particular, gene therapy strategies. He was coordinator of Epixchange and is currently PI in EPITARGET, both EC FP7 projects.

Affiliation
Università degli Studi di Ferrara and Vita-Salute San Raffaele, Milan

Project
Epixchange, EPITARGET

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Katja Kobow

**Biography**
Katja Kobow is an Assistant Professor at the Institute of Neuropathology at the Universitätsklinikum Erlangen, Friedrich-Alexander-Universität of Erlangen-Nürnberg in Germany. She received her PhD in 2009 under the supervision of Prof. Ingmar Blümcke and since then has built her own research group studying molecular pathomechanisms underlying experimental and human focal epilepsies. The main focus has been on epigenomic changes and the identification of molecular biomarkers for epilepsy and associated pathologies. Ongoing mechanistic studies address the translation of hypersynchronous neuronal activity, i.e. seizures, into aberrant epigenetic signatures and possibilities to target these mechanisms, e.g. through metabolic interference, both in-vitro and in-vivo.

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**Project**
DESIRE

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**Epigenetic biomarkers in FCDs**

Focal Cortical Dysplasia (FCD) is a major cause of drug-resistant focal epilepsy in children. Diagnosis and clinico-pathological classification remains, however, a challenging issue in daily practice.

We addressed the question, whether genome-wide DNA methylation and gene expression could be used to classify human FCD tissue. DNA methylomes and transcriptomes were generated from massive parallel sequencing in surgical FCD specimens, matched with other epilepsy and non-epilepsy controls.

Differential analysis discriminated three FCD subtypes in our cohort by specific methylation signatures thereby assigning molecular subtypes to previously proposed FCD phenotypes, e.g. FCD Ia, Ila and IIb. Pathology-specific gene expression signatures however could not be identified.

Deep sequencing analysis of a panel covering 53 mTOR pathway genes revealed mutations in only two patients with FCD Ila subtype, which is in line with previous studies reporting low frequency mTOR pathway-associated mutations. Also we found no particular enrichment of differential DNA methylation or gene expression in mTOR pathway related genes.

Our studies extend the evidence for disease-specific methylation signatures towards focal epilepsies in favour of an integrated clinico-pathological and molecular classification system of FCD subtypes incorporating genomic methylation.
Felix Rosenow

Biography
Felix Rosenow is Director of the Epilepsy Center Frankfurt Rhine-Main, Goethe University, Frankfurt, Germany, since 2015. Previously he led the Epilepsy Center Hessen, Philipps-Universität Marburg, Germany. Currently he serves as co-coordinator of the EpimiRNA-project, speaker of the LOEWE Center of Personalized Translational Epilepsy Research (CePTER) as member of the ILEA Commission on Epilepsy Surgery and as 2nd Vice President of the German Society for Clinical Neurophysiology and Functional Imaging (DGKN). His research interests include the pathophysiology and of epilepsy, translational approaches and clinical trials for epilepsy. He is member a number of scientific societies including ILAE, DGfE and DGN.

Affiliation
Goethe-Universität Frankfurt & Philipps-Universität Marburg

Project
EpimiRNA

MicroRNA as biomarker of epilepsy and status epilepticus

Brain-specific microRNA are suitable potential biomarkers. They are related to the pathophysiology of epilepsy, have known subcellular origins, are easily available and stable in different body fluids and can be quantified.

The objectives of EpimiRNA are to identify and evaluate microRNA based biomarkers (miR-BM). We assess in 3 different animal models which miR-BM are regulated during epileptogenesis, in epileptic animals and in epilepsy patients in comparison with control groups including patients with psychogenic seizures (PNES). To learn how and when mir-BM get from the brain into blood their expression in plasma and intracerebral microdialysate are correlated. Finally, we evaluate if therapeutic brain stimulation changes miR-expression and if these are predictive of therapeutic response.

Results of EpimiRNA are firstly: two specific exosomal miRNA in combination could discern Temporal Lobe Epilepsy (TLE)-patients from controls with an ROC-AUC of 0.87, while patients with PNES were not different from controls (Raoof et al. in preparation). Secondly: In CSF miRNAs 19b-3p, 21-5p and 451a combined were most suitable to differentiate TLE from other neurological diseases (ROC AUC=0.83) and status epilepticus from all other groups (ROC AUC=0.85; Raoof et al. Sci Rep. 2017). And finally, a miR-detector (TORNADO) was developed which can test miR-levels of different relevant miR-BM within 1.5 h in a bed-side manner (McArdle et al. Sci Rep. 2017).

Conclusions: MicroRNA seem to be valuable biomarkers for different stages of epileptogenesis. More importantly miR-BM may allow to confirm the diagnosis in patients with TLE and status epilepticus and to exclude clinically relevant differential diagnoses. Further validation studies are needed and under way.
Gianpiero Cavalleri

Biography
Gianpiero Cavalleri is Associate Professor of Human Genetics at the Royal College of Surgeons in Ireland and Deputy Director of the SFI FutureNeuro Research Centre. He completed his PhD studies in Genetics at University College London in 2005. After a brief Postdoctoral position at Duke University, he joined RCSI in 2006. His work is focused on the genetics of epilepsy predisposition and treatment, and he has contributed to several clinically relevant discoveries that have impacted the care of epilepsy.

Affiliation
Royal College of Surgeons in Ireland

Project
EpiPGX

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Predictors of adverse reactions to AEDs
The EpiPGX Consortium aims to identify genome-based biomarkers for use in clinical practice to individualise treatment of epilepsy. This talk will focus on collaborative efforts to map clinically relevant genetic factors that are predictive of adverse drug reactions. We have assembled a DNA cohort of over 1,000 people with epilepsy with AED-related adverse drug-reaction phenotypes that meet specific criteria. Dense genetic data in the form of GWAS and exome sequence is available on these patients.

An overview of results to date will be presented, including the role of genetic variation in complement factor H as a predictor of phenytoin-induced maculopapular exanthema.

Progress to date has been driven by close coordination within the community, but there is much work to be done. Opportunities and challenges relating to discovery in the research setting, and translation to the clinic will be discussed.
Lieven Lagae is Full Professor at the University of Leuven, Belgium (KUL) and is head of the Paediatric Neurology Department. He is the immediate past president of the European Paediatric Neurology Society (EPNS) and serves as Board Member of the International Child Neurology Association (ICNA). He chairs the Taskforce ‘Medical treatment of Childhood Epilepsy’ of the International League against Epilepsy (ILAE) and is also a steering committee member of the ERN EpiCare. Research projects include translational research in Zebrafish models of epilepsy, new anti-epileptic drugs and brain stimulation in childhood epilepsy and preventive treatment of epilepsy in tuberous sclerosis complex.

EEG changes as biomarker for emerging epilepsy in TSC

About 70% of all children with tuberous sclerosis complex (TSC) will develop epilepsy before the age of one year. In many cases this epilepsy is severe, difficult to control and associated with later intellectual disability, autism and behavioural problems. In the EPISTOP trial, we study whether preventive treatment with anti-epileptic drugs before the onset of clinical seizures will prevent or delay the onset of epilepsy.

Our hypothesis is that preventive treatment will be associated with a better global developmental outcome. We developed a unique scoring system for EEG classification to be used in all participating centres. Infants included in the randomized trial get EEGs every 4 weeks and whenever the EEG shows a specific EEG score (indicating epileptic activity), the infants will be randomised to preventive treatment (with vigabatrine) or standard treatment (= only AED treatment when clinical seizures occur).

Our preliminary findings show that the EEG in 96% of the infants with TSC show epileptic activity already in the first months of life. All these children were randomised and are now being followed for 2 years. We await the final results of RCT to see the effect of preventive treatment on global outcome. If preventive treatment would show a significant benefit, this would change our treatment paradigm in TSC substantially. In addition, this model of preventive AED treatment is applicable in other diseases associated with a high prevalence of epilepsy.
Annamaria Vezzani

Biography
Annamaria Vezzani is head of the laboratory of Experimental Neurology at Mario Negri Institute. The main research activities are devoted to characterising the molecular, biochemical and cellular mechanisms involved in the genesis and propagation of seizures in epileptic disorders, and the associated neurological co-morbidities, for finding more effective therapeutics with limited side-effects. She is an internationally recognised leading scientist in establishing the role of inflammatory pathways in the etiopathogenesis of seizures and excitoxicity, namely the IL-1 and Toll-like receptor signalling. Special focus of current research is on glio-neuronal communication in healthy and diseased brain, epigenetic mechanisms of glio-neuronal dysfunctions, molecular brain imaging and blood biomarkers.

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Mario Negri Institute for Pharmacological Research

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HMGB1 as a predictive biomarker for preclinical and clinical studies

We explored minimally invasive means to reliably predict the onset of epilepsy after brain injury in animal models to discover biomarkers that could increase the sensitivity of the existing clinical indicators. Our approaches include molecular brain imaging and the measure of soluble molecules in blood which may reflect brain intrinsic events involved in epilepsy development. Among these, we focused on Magnetic Resonance Spectroscopy (1H-MRS) based analysis of astrocytic activation, and the blood levels of the astrocyte-related and ictogenic inflammatory protein High Mobility Group Box 1 (HMGB1), since astrocytes appear to be pivotally involved in epileptogenesis triggered by brain insults. Moreover, we also investigated behavioral biomarkers of cognitive dysfunctions since this deficit represents a typical comorbidity in epilepsy which may manifest even before the onset of spontaneous seizures.

Our data show the prognostic value of both increased levels of myo-inositol in the hippocampus assessed by 1H-MRS as a marker of astrocyte’s activation and the associated cognitive deficits, which allowed early stratification of animals developing epilepsy. Blood HMGB1 levels also discriminate with high fidelity, and early after brain injury, between animals developing or not developing spontaneous seizures.

Our findings can be easily translated to the clinical setting for enriching the patient population to be recruited in clinical trials designed to study anti-epileptogenic treatments.

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Our findings can be easily translated to the clinical setting for enriching the patient population to be recruited in clinical trials designed to study anti-epileptogenic treatments.
2 Genetics of Epilepsy - Therapeutics Implications

Chairs & Speakers
Biographies & Abstracts
David Kwiatkowski
Chair Genetics

Biography
David Kwiatkowski is a Professor of Medicine at Harvard Medical School, Senior Physician at Brigham and Women’s Hospital/Dana-Farber Cancer Institute, leader of the Dana-Farber/Harvard Cancer Center Cancer Genetics program, Associate Member of the Broad Institute, and serves on numerous External Advisory Boards. Dr. Kwiatkowski has made major contributions to our understanding of Tuberous Sclerosis Complex (TSC) including: identification of TSC1, development of numerous mouse models of TSC, analysis of mosaicism in TSC, and development of targeted therapeutics. He has been recognised with research awards from both the Tuberous Sclerosis Alliance and the LAM Foundation.

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Project
EPISTOP

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Renzo Guerrini
Chair Genetics

Biography
Renzo Guerrini is an ordinary professor of Child Neuropsychiatry at the University of Florence and is Director of the Center of Neuroscience excellences of the Meyer AOU and of the School of Specialisation in Child Neuropsychiatry. His research focuses on neurophysiology, the neurogenetics of epilepsies and the malformations of the cerebral cortex. In 2003 he received the “Ambassador of Epilepsy” award from ILAE and in 2012 the “Epilepsy Research Recognition Award for Clinical Science” from the AES. He has participated or been coordinator of Commissions of International Organizations like WHO, ILAE, EMA and INSERM. Since 2013 he coordinates the European project “DESIRE - Development and Epilepsy”.

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Project
DESIRE

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Dr. Rima Nabbout is Professor of Paediatric Neurology at Paris Descartes University as well as Director of the Centre for Rare Epilepsies at Necker Enfants Malades Hospital, Imagine Institute, Paris, France. She is a member of the steering committee of EpiCARE (European reference network on rare epilepsies) and of scientific committees of patient’s associations on rare epilepsies. She received her medical degree from Saint Joseph University, Beirut, Lebanon; her paediatric board from Descartes University, Paris; and a PhD in Neurosciences from University Pierre et Marie Curie, Paris, France. Dr. Nabbout’s areas of research include electro clinical delineation of rare childhood epilepsies, genetics of epileptic encephalopathies, clinical trials in rare epilepsies and transition from childhood into adulthood. She has authored or co-authored more than 150 peer-reviewed papers, and is presently leading a research group at Inserm U1129 dedicated to “epilepsy in childhood and brain plasticity”

**Biography**

Dr. Rima Nabbout is Professor of Paediatric Neurology at Paris Descartes University as well as Director of the Centre for Rare Epilepsies at Necker Enfants Malades Hospital, Imagine Institute, Paris, France. She is a member of the steering committee of EpiCARE (European reference network on rare epilepsies) and of scientific committees of patient’s associations on rare epilepsies. She received her medical degree from Saint Joseph University, Beirut, Lebanon; her paediatric board from Descartes University, Paris; and a PhD in Neurosciences from University Pierre et Marie Curie, Paris, France. Dr. Nabbout’s areas of research include electro clinical delineation of rare childhood epilepsies, genetics of epileptic encephalopathies, clinical trials in rare epilepsies and transition from childhood into adulthood. She has authored or co-authored more than 150 peer-reviewed papers, and is presently leading a research group at Inserm U1129 dedicated to “epilepsy in childhood and brain plasticity”

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**Project**

EpiCARE

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**The emerging role of genetics in the clinical care pathway**

Genetics is transforming clinical practice in epilepsy, especially in rare and complex epilepsies. The epilepsy-associated genome is quickly growing with isolation of de novo variants in patients and targeted mutagenesis in models. The functional impact of detected variants in these models is revolutionising our understanding of the underlying mechanisms and makes the promise of precision therapies becoming closer to reality. Genetic diagnosis adds to the accuracy of the information. The diagnosis odyssey of families and patients comes to an end as well as false beliefs and self-blame. Genetic diagnosis allows also for specific counselling providing more precise information rather than broad empiric risk estimates. The reduced cost of genetic testing, the availability of gene panels, and whole exome or genome sequencing help genetics in becoming part of routine epilepsy practice, especially in paediatric settings.

Finally, analysis of epilepsy-associated genes moves us a step closer to further understanding of higher cortical functions mechanisms and neuronal synchronisation disorders.

Genetic diagnosis mainly avoids unnecessary testing with repeated complex blood tests, MRIs, invasive biopsies, pre-surgical workup, decreasing dramatically the health costs. Interestingly, in a growing number of genetic diagnoses specific indications in management are indicated. This may include the choice of conventional antiepileptic agents or the use of alternative therapies based on clinical experience or preclinical data.

A specific molecular diagnosis, when available, adds to the accuracy of the information. The diagnosis odyssey of families and patients comes to an end as well as false beliefs and self-blame. Genetic diagnosis allows also for specific counselling providing more precise information rather than broad empiric risk estimates. The reduced cost of genetic testing, the availability of gene panels, and whole exome or genome sequencing help genetics in becoming part of routine epilepsy practice, especially in paediatric settings.

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A specific molecular diagnosis, when available, adds to the accuracy of the information. The diagnosis odyssey of families and patients comes to an end as well as false beliefs and self-blame. Genetic diagnosis allows also for specific counselling providing more precise information rather than broad empiric risk estimates. The reduced cost of genetic testing, the availability of gene panels, and whole exome or genome sequencing help genetics in becoming part of routine epilepsy practice, especially in paediatric settings.
Iscia Lopes-Cendes is a physician-scientist, professor of Medical Genetics and head of the laboratory of Molecular Genetics at the department of Medical Genetics, School of Medical Sciences, University of Campinas (UNICAMP), Brazil. She works in the field of neurogenetics, focusing on the study of genetic and phenotypic markers in neurologic disorders. Currently, she is particularly interested in studying the underlying molecular mechanisms leading to disease, aiming to find better treatment options and prevention. In recent years, her laboratory has focused on the use of new genomic techniques in order to answer some of these biological and clinical questions.

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**Project**
EpimiRNA

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**Biography**

Iscia Lopes-Cendes is a physician-scientist, professor of Medical Genetics and head of the laboratory of Molecular Genetics at the department of Medical Genetics, School of Medical Sciences, University of Campinas (UNICAMP), Brazil. She works in the field of neurogenetics, focusing on the study of genetic and phenotypic markers in neurologic disorders. Currently, she is particularly interested in studying the underlying molecular mechanisms leading to disease, aiming to find better treatment options and prevention. In recent years, her laboratory has focused on the use of new genomic techniques in order to answer some of these biological and clinical questions.

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**Genetic variation in microRNAs and their targets in epilepsy**

The major goal of WP5 in EpimiRNA is to test whether human DNA sequence variation present in miRNAs, miRNA biosynthesis genes and 3’UTR miRNA target regions is associated with different epilepsy phenotypes. Patient recruitment was conducted at different clinical centres and phenotypes were divided into three main groups MTLE+HS, GGE and non-lesional FE. Genomic DNA was submitted to target enrichment using a customised gene panel and NGS was performed at Columbia University and at UNICAMP. For the analysis we implemented automated pipelines and methodologies for SNP and for Gene-based association as well as machine learning methods for genomic-based disease outcome prediction.

Preliminary analysis, on a subset of EpimiRNA samples, shows that the best model for classifying cases and controls resulted from a Random Forest built with 100 decision trees and 16 variables as the maximum depth. Applying a threshold of 10% for the cumulative percentage of variability explained by the model, we were able to select a subset of SNPs potentially associated with the phenotype. In addition, gene-based association analysis identified 10 genes possibly associated to the phenotype at p<0.05. We are currently performing additional analysis in the complete EpimiRNA dataset.
Holger Lerche

Biography
Prof. Dr. med. Holger Lerche is Clinical Director and Head of the Department of Neurology and Epileptology at the Hertie Institute of Clinical Brain Research at the University of Tübingen, Germany. He was a consultant neurologist and Head of Epileptology in the Department of Neurology at the University of Ulm from 2001 to 2009, being a Heisenberg fellow of the DFG from 2003 to 2008 during which he also undertook research fellowships in London and Melbourne, before taking up his current position in Tübingen. His main research interest is to unravel the genetics and pathophysiology of inherited epilepsies and related paroxysmal disorders using a combination of genetic and neurophysiological tools. He is leading a DFG Research Unit ‘Epileptogenesis of Genetic Epilepsies’. Holger Lerche is Chair of the ILAE Genetics Commission and of Editorial Board member of Epilepsia, and a former president of the German chapter of the ILAE.

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Project
EpiPGX

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Predictors of responses to AEDs
Up to date, pharmacotherapy of the epilepsies is guided by trial and error, since predictors for a response to specific antiepileptic drugs is largely missing. Recent advances in this field come from genetics, offering specific established and new therapies for genetic epilepsy syndromes based on pathophysiological principles.

Examples are provided by variants in ion channel or transporter encoding genes which cause a broad spectrum of epilepsy syndromes of variable severity and onset. (1) the ketogenic diet for glucose transporter defects of the blood-brain barrier, (2) Na⁺ channel blockers (e.g. carbamazepine) for gain-of-function Na⁺ channel mutations and avoidance of those drugs for loss-of-function mutations, and (3) specific K⁺ channel blockers for mutations with a gain-of-function defect in respective K⁺ channels. But also in other areas, specific pathways can be therapeutically relevant, such as recently shown for mTOR inhibitors in tuberous sclerosis.

For the more common epilepsies, like genetic generalized or focal epilepsies, we have been looking for pharmacogenetic markers to predict responses for specific AEDs in the frame of the EpiPGX project, but so far cannot offer such markers. In conclusion, genetics offer promising tools to predict therapeutic effects in the epilepsies, but further work is needed to use this knowledge for a broad spectrum of the epilepsies.
David Kwiatkowski

Biography
David Kwiatkowski is a Professor of Medicine at Harvard Medical School, Senior Physician at Brigham and Women’s Hospital/Dana-Farber Cancer Institute, leader of the Dana-Farber/Harvard Cancer Center Cancer Genetics program, Associate Member of the Broad Institute, and serves on numerous External Advisory Boards. Dr. Kwiatkowski has made major contributions to our understanding of Tuberous Sclerosis Complex (TSC) including: identification of TSC1, development of numerous mouse models of TSC, analysis of mosaicism in TSC, and development of targeted therapeutics. He has been recognized with research awards from both the Tuberous Sclerosis Alliance and the LAM Foundation.

Affiliation
Harvard Medical School

Project
EPISTOP

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Genetics and mosaicism in Tuberous Sclerosis Complex: preliminary analysis of association with seizure phenotype in EPISTOP

Tuberous sclerosis complex (TSC) is due to inactivating mutations in either TSC1 or TSC2. 101 TSC infants were enrolled into EPISTOP, of which 3 did not meet diagnostic criteria for TSC. Probable or definite mutations in TSC1 or TSC2 have been identified in 93 of 98 (95%) subjects. 23 (25%) mutations are in TSC1 while 70 (75%) are in TSC2. The types of mutation were: 8 (9%) insertions, 17 (18%) deletions, 17 (18%) splice, 21 (23%) missense, 21 (23%) nonsense, 7 (7%) large deletions, and 2 (2%) in-frame deletions. Eight (of 93, 9%) subjects have mosaic mutations, at allele frequencies ranging from 0.7% to 18%.

Our findings of mutations in 95% of subjects is clearly a very high mutation detection rate, and is similar to other recent series using massively parallel sequencing, and comprehensive analysis to search for TSC mutations. The 9% frequency of mosaic mutations is somewhat surprising, especially the 3 subjects with allele frequencies < 5% (0.7%, 3.0%, 4.4%), who still meet diagnostic criteria with multi-system involvement by age 3 months. This likely reflects that the mosaic allele frequency is different in different tissues and organ systems. Further study is underway.
Albert Becker

**Biography**

Albert Becker is Professor for Molecular Neuropathology and Head of the Section for Translational Epilepsy Research at the Department of Neuropathology in the University of Bonn. The major scientific interests of his group are on basic pathomechanisms of epilepsies. His group hosts one of the largest fresh-frozen human brain tissue banks from biopsies of pharmacoresistant epilepsy patients undergoing neurosurgery for seizure relief. Complementarily, they apply epilepsy model systems of different complexity. On this joint basis he contributes to translation of data derived from animal models into the human disease context as a major goal of EPITARGET to create new biomarkers and treatments for epilepsy.

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**Project**

EPITARGET

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Functional epigenomic dissection of epilepsy in human brain biopsy tissue and corresponding mouse models

Genetic and acquired epilepsies can affect identical target molecules, in particular in form of mutational versus transcriptional ‘ion-channelopathies’. In the first context, rare deleterious gene mutations and common polygenic variation act synergistically and causal SNPs in non-coding regions exert a regulatory effect on the transcriptional activity of adjacent genes by an allelic functional alteration of cis-acting epigenetic elements.

In the second, dynamic epigenetic regulation after brain insults including status epilepticus (SE) orchestrates transcriptional dynamics that finally result in a hyperexcitable focus such as in Temporal Lobe Epilepsy (TLE). Understanding epigenetic regulation of key epileptogenesis molecules including the pore-forming Ca2+ channel subunit Cav3.2 that mediates a low voltage-activated (T-type) Ca2+ current ($I_{CaT}$) contributing pivotally to neuronal activity is therefore mandatory.

Hippocampal biopsies from pharmacoresistant Temporal Lobe Epilepsy patients represent a unique resource to gain insights in epigenetic gene regulation in the human brain. Data will be presented for Cav3.2 on how experiments starting from these unique human brain tissue and transgenic mouse models are combined to understand epigenetic regulation in focal as well as generalised epilepsies.
3 Innovation Therapeutics & Translation

Chairs & Speakers
Biographies & Abstracts
Felix Rosenow
Chair Therapeutics

Biography
Felix Rosenow is Director of the Epilepsy Center Frankfurt Rhine-Main, Goethe University, Frankfurt, Germany, since 2015. Previously he led the Epilepsy Center Hessen, Philipps-Universität Marburg, Germany. Currently he serves as co-coordinator of the EpimiRNA-project, speaker of the LOEWE Center of Personalized Translational Epilepsy Research (CePTER) as member of the ILEA Commission on Epilepsy Surgery and as 2nd Vice President of the German Society for Clinical Neurophysiology and Functional Imaging (DGKN). His research interests include the pathophysiology and of epilepsy, translational approaches and clinical trials for epilepsy. He is member a number of scientific societies including ILAE, DGF and DGfE.

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Mérab Kokaia
Chair Therapeutics

Biography
Professor Mérab Kokaia is head of the Epilepsy Center at Lund University, and coordinator of EPITARGET. His research focuses on neuropeptides and neurotrophic factors as strong regulators of synaptic transmission in the CNS, offering a potent tool to counteract seizure activity. Kokaia’s group uses rodent epilepsy models (kindling, post-status epilepticus) and in vitro approaches (patch-clamp in brain slices, optogenetics, chemogenetics). One of the specific aims is direct and indirect gene transfer for various factors into the brain, including optogenetics and chemogenetics, to investigate mechanisms of ictogenesis and epileptogenesis, and develop new gene therapy-based strategies for epilepsy treatment.

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EPITARGET

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Dr. Benfenati’s main subjects of investigation have been the mechanisms of synaptic communication in health and experimental models of synaptopathies. More recently he has extended his investigations to neuronal network engineering by exploiting optogenetics and photovoltaic interfaces for neuronal modulation. The double background of neurologist and cellular neurophysiologist allowed him to get in depth into the cellular mechanisms that underlie normal brain function and whose dysfunction leads to the pathogenesis of neurological diseases. He has been involved in several EU and national research projects such as MSCA ITN “Extracellular Matrix in Epileptogenesis”, H2020 Flagship Graphene and DESIRE.

**Innovative therapeutic strategies for epileptic encephalopathies**

DESIRE proposed to develop innovative therapeutic strategies for treating and preventing epilepsy-onset in children with Epileptic Encephalopathies. To this aim, innovative genetically encoded probes to counteract the changes leading to excitation/inhibition imbalance and hyperexcitability and a cell therapy for treating refractory epilepsy were generated. Seizure activity is associated with intracellular and extracellular pH drops that may further increase neuronal excitability. To keep neuronal activity under control, we generated a luciferase-driven E2-GFP pH sensor fused with halorhodopsin, which actuates membrane hyperpolarisation in response to a drop in intracellular pH.

Previous data demonstrated an altered expression of the transcription repressor REST in epilepsy which might play a major role in epileptogenesis. We have engineered an optogenetically driven REST inhibitor that releases REST target genes from inhibition in response to light stimulation. The probe, expressed in vivo via the administration of AAV-carried AsLOV2-PAH1, releases REST target genes from inhibition and ameliorates kainic acid-induced seizures.

We also demonstrated that, through the overexpression of only a minimal set of specific transcription factors, it is possible to convert adult somatic cells such as dermal fibroblasts into GABAergic inhibitory neurons that can replace dead or degenerated GABAergic neurons, thus delaying and restraining hyperexcitability. Future preclinical investigations are required for demonstrating the efficacy of these strategies to prevent or cure epilepsy in relevant animal models before a translation to human patients is envisioned.
Emilio Perucca

Biography
Emilio Perucca is currently Professor at the University of Pavia and Director of the Clinical Trial Centre at the Mondino Neurological Institute in Pavia, Italy. He is trained as a neurologist and clinical pharmacologist at the National Hospital for Nervous Diseases, London and is the immediate Past-President of ILAE and the recipient of the 2018 European Epileptology Award. His research activities, which contributed to over 450 Pubmed-listed publications, have focused on the drug treatment of seizure disorders and outcome assessment of outcome in people with epilepsy. He was coordinator of the pharmacogenomic Workpackage in the EU-funded EPICURE project.

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Project
EpiCARE

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Improving clinical trials in epilepsy: addressing the hurdles and filling the gaps

Rational decisions concerning medical interventions should be based on adequate evidence about the benefits and risks of available treatment options, which can only be derived from well-designed clinical trials and, particularly, randomised controlled trials (RCTs).

A critical review of available evidence, however, reveals the majority of RCTs in epilepsy have been designed to address regulatory requirements, and provide information of limited value to guide physicians in their clinical practice. Moreover, there is a paucity of RCTs in epilepsy syndromes other than focal epilepsies, and no RCTs at all in most of the less common syndromes of infancy and childhood.

In the light of these shortcomings, there is scope to (i) reassess regulatory requirements to facilitate generation of data more directly applicable to the routine clinical setting; (ii) develop innovative trial designs with more clinically meaningful endpoints, and with minimised exposure to placebo; (iii) select (or stratify) patients based on specific syndromic phenotypes and/or specific etiologies; (iv) include whenever feasible an optimally used active control.

Funding organisations should be sensitised to the current lack of adequate evidence to guide therapeutic practice in epilepsy, and the need to promote high quality comparative effectiveness trials. Future therapeutic trials, including pragmatic trials, in Europe will benefit from the active collaboration being developed within EpiCARE, the European Reference Network dedicated to rare and complex epilepsies.
David Henshall is professor of molecular physiology & neuroscience at the Royal College of Surgeons in Ireland (RCSI). After his PhD in the UK in 1997, he moved to the USA for post-doctoral training at the University of Pittsburgh. He established his first lab at a private research institute in Portland, Oregon in 1999 before moving to RCSI in 2004. His research interests are the cell and molecular mechanisms of epilepsy. He has authored over 160 papers and book chapters and is Director of FutureNeuro, the Irish research centre for chronic and rare neurological diseases. He is Coordinator of EpimiRNA.

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Project
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Biography
David Henshall is professor of molecular physiology & neuroscience at the Royal College of Surgeons in Ireland (RCSI). After his PhD in the UK in 1997, he moved to the USA for post-doctoral training at the University of Pittsburgh. He established his first lab at a private research institute in Portland, Oregon in 1999 before moving to RCSI in 2004. His research interests are the cell and molecular mechanisms of epilepsy. He has authored over 160 papers and book chapters and is Director of FutureNeuro, the Irish research centre for chronic and rare neurological diseases. He is Coordinator of EpimiRNA.

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MicroRNAs as targets for seizure control and disease-modification

A major objective of the EpimiRNA consortium is to develop novel approaches for seizure control and disease-modification in adult forms of intractable Temporal Lobe Epilepsy. This talk will provide an overview of EpimiRNA's therapeutics-focused research which has focused on small noncoding RNAs called microRNAs.

First, progress toward pre-clinical development of a previously-identified brain-enriched microRNA. EpimiRNA's research has shown that delivery of an oligonucleotide inhibitor (antimir) of microRNA-134 shortly after an experimental status epilepticus dramatically reduces the later occurrence of spontaneous recurrent seizures in multiple rodent models. Studies have also uncovered the primary molecular mechanism by which these effects are mediated.

Second, EpimiRNA has undertaken systematic screens of a set of ten antimirs targeting microRNAs commonly regulated in models of status epilepticus. This has identified five additional microRNAs that can be targeted for seizure-suppression or neuroprotection.

Finally, in collaboration with one of EpimiRNA's SMEs, we have identified anti-seizure actions of a new small molecule in experimental and human models of epilepsy. Together, these studies represent important advances in our understanding of the therapeutic potential of targeting microRNAs for the treatment or prevention of epilepsy. To move one or more of these microRNA-based therapies forward will require partnering with industry, addressing questions around efficacy in human tissue, how to identify and select suitable patients, toxicity-safety of these molecules in the brain and practical challenges of how such large molecules could be given to patients.
Sergiusz Jóźwiak

Biography
Sergiusz Jóźwiak is a Professor and the Head of the Department of Paediatric Neurology at the Warsaw Medical University. From 1995-2015 he held the position of the Head of the Paediatric Neurology and Epileptology Department at the Children's Memorial Health Institute. Prof. Jóźwiak's research focuses mainly on neurocutaneous disorders and epilepsy, especially infantile spasms. Currently, he is a coordinator of the large-scale European Commission Project EPISTOP evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy—tuberous sclerosis complex (www.epistop.eu). Prof. Jóźwiak is an active member of numerous international organisations, and has published more than 300 papers in peer reviewed journals. Prof. Jóźwiak is on the editorial boards of several professional journals as Pediatric Neurology, European J Paediatric Neurology, Journal of Child Neurology.

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Project
EPISTOP

Preseizure vs postseizure treatment of epilepsy - pros and cons

Despite a great progress in the management of epilepsy, about 50% of children with epilepsy suffer from epilepsy-related co-morbidities, including developmental delay and autism spectrum disorder. The EU-funded EPISTOP is the first prospective study of epileptogenesis in humans, starting before seizures and expanding beyond the point when drug-resistant epilepsy and epilepsy co-morbidities can be assessed. The project is conducted in children with tuberous sclerosis complex (TSC) considered as an excellent clinical model of severe focal epilepsy.

The primary clinical endpoint of this study will be the decrease in epilepsy severity and the clinically active epilepsy incidence in the preventatively treated group in comparison to the group treated after the onset of clinical seizures. The secondary endpoints will be the changes in biomarkers between two groups.

Altogether EPISTOP will provide insights into novel diagnostic laboratory tests to predict epilepsy onset, and clinical recommendations to identify patients at high risk of epilepsy development before epilepsy is clinically active.

The final results will be known after study completion, however, preliminary analysis are promising. The proportion of normal EEGs dramatically decreases with age, with only maximum 20% normal EEGs above the age of 12 months. However, children treated before clinical seizures occurrence seem to develop clinical seizures later in comparison to standard care group (treated after the onset of seizures). Other analysis including molecular, imaging studies and neuropsychological analysis are ongoing. The project is coordinated by the Children's Memorial Health Institute and 16 partners from Europe, Australia and USA.
Wolfgang Löscher

**Biography**

Wolfgang Löscher is Professor and Director of the Department of Pharmacology, Toxicology and Pharmacy as well as Head of the Center for Systems Neuroscience in Hannover, Germany. His main area of interest is epilepsy research, particularly preclinical development of new treatments, and he is an internationally renowned expert in this field. Löscher’s laboratory developed the first animal models of drug resistant epilepsy and is using and providing these models in the search for new therapeutic strategies. Furthermore, his group studies mechanisms of epileptogenesis and how to prevent or modify epilepsy by interfering with these mechanisms.

**Affiliation**

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**Project**

EPITARGET

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**Evaluation of tolerability and antiepileptogenic efficacy of multitargeted drug combinations by a two-stage approach**

Prevention of epilepsy (“antiepileptogenesis”) in patients at risk is a major unmet clinical need. Several drugs underwent clinical trials for epilepsy prevention, but none of the drugs tested were effective. Similarly, most previous preclinical attempts to develop antiepileptogenic strategies failed. In the majority of studies, drugs were given as monotherapy.

However, epilepsy is a complex network phenomenon, so it is unlikely that a single drug can halt epileptogenesis. We recently proposed multitargeted approaches (“network pharmacology”) to interfere with epileptogenesis. One strategy, which, if effective, would allow a relatively rapid translation into the clinic, is developing novel combinations of clinically used drugs with diverse mechanisms that are potentially relevant for antiepileptogenesis. In order to test this strategy preclinically, we developed a preclinical two-stage approach for testing such drug combinations, which was inspired by the established drug development phases in humans.

As a first step of this approach, tolerability of rationally chosen, repeatedly administered drug combinations is evaluated by a large test battery in small groups of mice, including experiments in which the drugs are given in the latent phase after status epilepticus. As a next step, the rationally chosen drug combinations are evaluated for antiepileptogenic activity in larger animal groups in mouse and rat models of acquired epilepsy. Promising data obtained by this strategy will be presented.
Marco Ledri

Biography
Marco Ledri is Assistant Researcher in Neurosciences at Epilepsy Center, Department of Clinical Sciences, Lund University. His research focuses on the development of new gene therapy strategies against epilepsy. Previously, he worked on the effects of neuropeptides, such as NPY and galanin, on synaptic transmission in mouse models and human epileptic tissue. He has also contributed to the investigation of the potential of optogenetic interventions for blocking epileptiform activity and seizures. Lately, his research focuses on the development of CRISPR/Cas9-based gene therapy approaches against epileptogenesis. He was involved in several EU-funded projects, including Neurotrain, Epicure, EpiXchange and more recently EPITARGET.

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Project
EpiXchange

Differential effect of neuropeptides on excitatory synaptic transmission in human epileptic hippocampus

Development of novel disease-modifying treatment strategies for neurological disorders, which at present have no cure, represents a major challenge for today’s neurology. Translation of findings from animal models to humans represents an unresolved gap in most preclinical studies.

Gene therapy is an evolving innovative approach that may prove useful for clinical applications. In animal models of Temporal Lobe Epilepsy (TLE), gene therapy treatments based on viral vectors encoding NPY or galanin have been shown to effectively suppress seizures. However, how this translates to human TLE remains unknown.

A unique possibility to validate these animal studies is provided by a surgical therapeutic approach, whereby resected epileptic tissue from temporal lobes of pharmacoresistant patients are available for neurophysiological studies in vitro. To test whether NPY and galanin have antiepileptic actions in human epileptic tissue as well, we applied these neuropeptides directly to human hippocampal slices in vitro. NPY strongly decreased stimulation-induced EPSPs in dentate gyrus and CA1 (up to 30 and 55%, respectively) via Y2 receptors, while galanin had no significant effect. Receptor autoradiographic binding revealed the presence of both NPY and galanin receptors, while functional receptor binding was only detected for NPY, suggesting that galanin receptor signaling may be impaired.

These results underline the importance of validating findings from animal studies in human brain tissue, and advocate for NPY as a more appropriate candidate than galanin for future gene therapy trials in pharmacoresistant TLE patients.
4 Understanding Co-Morbidities in the Epilepsies

Chairs & Speakers
Biographies & Abstracts
Professor Helen Cross is The Prince of Wales’s Chair of Childhood Epilepsy and Honorary Consultant in Paediatric Neurology at UCL-Great Ormond Street Institute of Child Health, as well as Head of the Developmental Neuroscience Programme, London and Young Epilepsy, Lingfield, UK. Her research has been targeted at improving outcomes in early onset epilepsy, specifically in assessing the role of surgery and ketogenic diet. She is elected Treasurer of the International League Against Epilepsy 2017-2021, Clinical Advisor to the National Children’s Epilepsy Surgery Service, Chair of the BPNA Research Committee, Chair of the Neurosciences Clinical Study Group of the Clinical Research Network (Children) and coordinator of the European Reference Network for rare and complex epilepsies, EpiCARE. She holds NIHR Senior Investigator status 2016-2020. She was awarded ILAE/IBE Ambassador for Epilepsy in 2007, and an OBE in the Queen’s Birthday Honours in 2015.

Sergiusz Jóźwiak is a Professor and the Head of the Department of Paediatric Neurology at the Warsaw Medical University. From 1995-2015 he held the position of the Head of the Paediatric Neurology and Epileptology Department at the Children’s Memorial Health Institute. Prof. Jóźwiak’s research focuses mainly on neurocutaneous disorders and epilepsy, especially infantile spasms. Currently, he is a coordinator of the large-scale European Commission Project EPISTOP evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy –tuberous sclerosis complex (www.epistop.eu). Prof. Jóźwiak is an active member of numerous international organisations, and has published more than 300 papers in peer reviewed journals. Prof. Jóźwiak is on the editorial boards of several professional journals as Pediatric Neurology, European J. Paediatric Neurology, Journal of Child Neurology.

Helen Cross
Chair Co-Morbidities

Biography
Professor Helen Cross is The Prince of Wales’s Chair of Childhood Epilepsy and Honorary Consultant in Paediatric Neurology at UCL-Great Ormond Street Institute of Child Health, as well as Head of the Developmental Neuroscience Programme, London and Young Epilepsy, Lingfield, UK. Her research has been targeted at improving outcomes in early onset epilepsy, specifically in assessing the role of surgery and ketogenic diet. She is elected Treasurer of the International League Against Epilepsy 2017-2021, Clinical Advisor to the National Children’s Epilepsy Surgery Service, Chair of the BPNA Research Committee, Chair of the Neurosciences Clinical Study Group of the Clinical Research Network (Children) and coordinator of the European Reference Network for rare and complex epilepsies, EpiCARE. She holds NIHR Senior Investigator status 2016-2020. She was awarded ILAE/IBE Ambassador for Epilepsy in 2007, and an OBE in the Queen’s Birthday Honours in 2015.

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Project
EpiCARE

Sergiusz Jóźwiak
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Biography
Sergiusz Jóźwiak is a Professor and the Head of the Department of Paediatric Neurology at the Warsaw Medical University. From 1995-2015 he held the position of the Head of the Paediatric Neurology and Epileptology Department at the Children’s Memorial Health Institute. Prof. Jóźwiak’s research focuses mainly on neurocutaneous disorders and epilepsy, especially infantile spasms. Currently, he is a coordinator of the large-scale European Commission Project EPISTOP evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy –tuberous sclerosis complex (www.epistop.eu). Prof. Jóźwiak is an active member of numerous international organisations, and has published more than 300 papers in peer reviewed journals. Prof. Jóźwiak is on the editorial boards of several professional journals as Pediatric Neurology, European J. Paediatric Neurology, Journal of Child Neurology.

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Project
EPISTOP
Alfonso Represa

**Biography**
Alfonso Represa, MD (University of Valladolid, Spain), PhD (University Paris VI, France), is Director of Research at the CNRS and former director of the INMED, an INSERM/ Aix-Marseille University reference center for Neuroscience and Epilepsy. He investigates Malformations of Cortical Development, which account for 20-40% of drug-resistant childhood epilepsy with the aim of elucidating the physiopathological mechanisms responsible for epileptogenesis, especially evaluating functional and structural alterations. His ultimate goal is to provide new therapeutic vistas. Dr. Represa has coordinated with success number of projects and published more than 110 publications in peer-reviewed journals (cited on average 57,22 times).

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DESIRE

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**Perspectives for investigating physiopathological mechanisms of co-morbidities in epilepsy**

Co-morbidities described in epilepsy, including psychiatric disorders, cognitive disorders, migraine, and sleep disorders, disturb the quality of life of patients and families but its causative mechanisms remain elusive. Different hypotheses, not necessarily exclusive, are proposed. They can result from: the gene mutation that causes the epileptic condition; a different genetic or environmental insult (a second hit); the epileptic activity itself that alters the normal operation of cortical networks; plastic functional/structural modifications of neurons and neuronal networks; a side effect of therapy. These hypotheses can be studied in appropriate animal models but past research did not really focus the investigation of causative mechanisms of co-morbidities. These investigations provided however some indications that will help orient our future research; some of these data will be presented here.

Animal models (essentially murine genetic models) investigations show that co-morbidities can be caused by a diversity of mechanisms that are related to the epileptic condition, from the original genetic cause (the same gene defect induces epilepsy and comorbid illness) to the epileptic activity per se. These investigations help to understand some of the pathomechanisms of comorbid syndromes. A way forward can be described as follows: Physiopathology of comorbid syndromes investigation is in its infancy and needs support.

Our investigations will help earlier diagnosis and will develop appropriate strategies for prevention, monitoring and treatment of patients with epilepsy and co-morbidities.

There are many opportunities to evaluate co-morbidities in rodent brains, but species differences, particularly in terms of cognitive functions, represent a challenge and additionally stress the need for reinforcing translational research.
Dr. Costin Leu is a senior computational biologist, who has gained worldwide recognition by co-authoring three out of all seven published genome-wide association studies (GWAS) in epilepsy (GWASs are listed in his review PMID: 26886358). In addition, he is one of the leading analysts of the ongoing Epi25 and ILAE Consortium on Complex Epilepsies projects, the two largest epilepsy projects. His main current research interest is the integration of multi-variant genomics and clinical data.

**Predictors of response and resistance and co-morbidities**

Co-morbidities represent additional clinical burden for people with epilepsy. Whilst genetic overlap between neurological disorders (NDs) and epilepsy has been explored, the individual genetic burden for each patient, and any contribution or complication it might create to genetic analyses and treatment solutions in epilepsy have been poorly explored. We tested in a sample of 3934 epilepsy patients with known drug therapy response status (2354 drug-resistant; 1580 responder), the hypothesis that genetic predispositions for NDs, represented by polygenic risk scores (PRS), will not be randomly distributed, so that groups of patients can be identified with similar and different patterns of PRS.

The main results are: K-means clustering in a matrix of normalized PRS for schizophrenia, Alzheimer’s, depression, attention-deficit hyperactivity disorder, and migraine identified three distinct cluster of patients. One of the cluster, representing patients with higher genetic predispositions for schizophrenia and Alzheimer’s, emerged as enriched with patients who did not respond to drug therapy (Fisher’s P=0.002; OR=1.27). This leads to the following impact: Genetic predispositions for NDs have, when combined, the potential to predict each epilepsy patient’s response to drug therapy.

Therefore, confirmatory analyses, using additional epilepsy patients with a known drug-response, combined with PRS derived from more powered ND-meta-analyses are needed, in order to not only confirm the results, but also to explore the existence a deeper structure beyond the three identified cluster of patient with a similar genetic risk makeup.
Paolo Curatolo

Biography
Paolo Curatolo is full professor of Paediatric Neurology and Psychiatry and Head of Paediatric Neuroscience Unit, University of Rome Tor Vergata, Italy. He has published 3 monographs and over 300 articles on child neurology, Tuberous Sclerosis Complex, epilepsy, autism, and ADHD (H-index 59). He served as President of the International Child Neurology Association (ICNA, 2002-2006) and has been for 20 years member of the Executive Board of the EFNS and of the ICNA. He has been awarded with Gomez Award by Tuberous Sclerosis Alliance in 2015. He is responsible for the work package concerning neurocognitive and developmental evaluations in the EPISTOP project.

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Project
EPISTOP

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Autism in Tuberous Sclerosis Complex: early developmental trajectories, potential biomarkers and treatment strategies

Autism Spectrum Disorders (ASD) are much more frequent in Tuberous Sclerosis Complex (TSC) than in the general population, affecting about 30-50% of patients. Despite this strong association, the exact mechanisms underlying this comorbidity are still largely unknown, but there is an obvious role played by early onset seizures, which confer a higher risk of developing ASD symptoms later in life.

Early autistic traits can be identified already in the first year of life, with a deviation from expected developmental trajectories, and alterations of playing, social interaction and eye gaze. After the second year of life, abnormal behaviours including hyperactivity, repetitive behaviours, and temper tantrums can appear. A slowing of development in the verbal area in the first years of life could predict the subsequent diagnosis of ASD. Children with TSC and ASD usually show lower cognitive abilities when compared to children with TSC but without ASD, and this difference is already evident at 12 months of age.

The importance of an early identification of ASD in children under the age of 2 years is crucial to initiate an early treatment that could ameliorate its disabling effects by making use of the greater brain plasticity of the early ages. Research studies in animal models of syndromic ASD revealed that enhanced environmental stimulations could ameliorate the effects of a wide range of neurological challenges.

Therefore, it is essential to identify predictive preclinical markers in infants with TSC aged 6-12 months, and this is exactly the aim of WP7 in the context of the EPISTOP project.
Michael Johnson

Biography
Michael Johnson is Professor of Neurology and Genomic Medicine at Imperial College London, and Honorary Consultant Neurologist at Imperial College Healthcare London. His research focuses on the systems-level integration of genetic, genomic and phenotypic data to identify causal functional networks and pathways underlying disease. Using systems-level approaches, disease-associated functional networks are then used to identify novel therapeutic targets and drugs which cannot otherwise be captured using traditional genetic strategies. He is a member of the ILAE Task Force on Genetics and Epigenetics, the ILAE Task Force on Pharmacogenetics, the ILAE Consortium on Complex Genetics, and the Epi4K, EpiPGX and EPITARGET international consortia.

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Systems genetic evidence for a convergence of epilepsy and its co-morbidities on shared molecular pathways

The genetic epilepsies are a heterogeneous group of disorders. Whilst the primary manifestation of epilepsy is recurrent seizures, epilepsy is also frequently associated with cognitive and psychiatric symptoms. Using an integrative systems biology approach, we have shown that genetic risk for epilepsy, healthy human intelligence and broad neuropsychiatric disease (autism, intellectual disability, schizophrenia) converges on a shared gene regulatory network enriched for synaptic functions (Johnson, et al. Nat. Neurosci. 2016;19:223–32).

These findings suggest many of the so-called “co-morbidities” of epilepsy are in fact simply a broader manifestation of the underlying biology of epilepsy, arguing for a broader conception of the epilepsy condition beyond simply recurrent epileptic seizures.
Chiara Falcicchia

Biography
Chiara Falcicchia received her Master Degree in Medical and Pharmaceutical Biotechnology in 2010 and her PhD in Pharmacology and Molecular Oncology in 2015, both from the University of Ferrara. As part of her doctorate research, she worked in two biotechnology companies, Iliviron of Lion, France and NsGene of Providence, USA. The main focus of her research is on cell and gene therapy for epilepsy. She is currently a postdoctoral fellow in the research group of Prof. Michele Simonato, at the Department of Medical Sciences of the University of Ferrara.

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Project
Epixchange

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Effects on seizures and epilepsy co-morbidities of encapsulated BDNF-producing cells

Brain-derived neurotrophic factor (BDNF) may represent a therapeutic for chronic epilepsy, but evaluating its potential is complicated by difficulties in its delivery to the brain. We will describe the effects on epileptic seizures of encapsulated cell biodelivery (ECB) devices filled with genetically modified human cells engineered to release BDNF. These devices, implanted into the hippocampus of pilocarpine-treated rats, decreased the frequency of spontaneous seizures by more than 80%.

This effect associated with improved cognitive performance, as epileptic rats treated with BDNF performed significantly better on a novel object recognition test. Detailed immunohistochemical analyses revealed that positive neurological effects were associated with several anatomical changes, including reduction in degenerating cells and increased neurogenesis and neuronal counts (including parvalbumin positive interneurons).

These data suggest that BDNF, when continuously released in the epileptic hippocampus, reduces the frequency of generalised seizures, improves cognitive performance, and reverts histological alterations associated with chronic epilepsy. Thus, ECB device-mediated long-term supplementation of BDNF in the epileptic tissue may represent a valid therapeutic strategy against epilepsy and some of its co-morbidities.

This approach may be directly applicable to patients selected for surgical resection of the epileptic hippocampus that undergo implantation of depth electrodes to define the epileptogenic area before surgery. ECB device(s) may be implanted together with these electrodes: if ineffective, they would be removed and the patient would undergo surgery as originally planned; if effective, the patient would have the option of avoiding surgery.
5 Biobanks and Databases – Basis for Translational Research

Chairs & Speakers
Biographies & Abstracts
Prof. Sisodiya’s laboratory works on epilepsy genetics, focusing on causes, treatment resistance, and pharmacogenomics. The methods include population genetics and studies of families and trios, complemented by deep phenotyping. The aim is to translate such findings directly into person-centred treatment. He’s already taking discoveries into early treatment options, facilitated by embedding research in a clinical context. Much of the work happens at the Epilepsy Society site in Chalfont, where co-location of a highly-specialised clinical evaluation facility and research laboratories drives patient-focused management. He leads a European FP7 Consortium on epilepsy pharmacogenomics (www.epipgx.eu) and works within other Consortia such as Euro EPINOMICS RES (www.euroepinomics.org).

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Sanjay Sisodiya
Chair Biobanks and Databases

Katja Kobow is an Assistant Professor at the Institute of Neuropathology at the Universitätsklinikum Erlangen, Friedrich-Alexander-Universität of Erlangen-Nürnberg in Germany. She received her PhD in 2009 under the supervision of Prof. Ingmar Blümcke and since then has built her own research group studying molecular pathomechanisms underlying experimental and human focal epilepsies. The main focus has been on epigenomic changes and the identification of molecular biomarkers for epilepsy and associated pathologies. Ongoing mechanistic studies address the translation of hypersynchronous neuronal activity, i.e. seizures, into aberrant epigenetic signatures and possibilities to target these mechanisms, e.g. through metabolic interference, both in-vitro and in-vivo.

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Project
DESIRE

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Katja Kobow
Chair Biobanks and Databases

Biography

Biography
Professor Blümcke is Director of the Dept. of Neuropathology at the University Hospital Erlangen (Germany). His research addresses clinical and molecular neuropathology of human epilepsy brain tissue specimens. Dr. Blümcke founded the European Epilepsy Brain Bank in 2006, which comprises up to date 10,000 epilepsy surgery specimen. Other achievements of his scientific carrier include a first international consensus classification of Focal Cortical Dysplasias (2011) and of Hippocampal Sclerosis (2013). His scientific work is documented by more than 270 peer-reviewed articles with a current h-factor of 58. Dr. Blümcke’s research is continuously funded by the EU-FP6 (LSHM CT-2006-037315), -FP7 (GA#602531) and -H2020 (GA#769051) health programmes.

Success of the European Epilepsy Brain Bank

Standardised collection of anatomically and pathophysiologically well characterised human brain tissues are required to identify disease-related pathomechanisms and new therapeutic targets. The European Epilepsy Brain Bank (EEBB) was founded in 2006 under the direction of EpiCure (FP6), continued in FP7 (DESIRE), and H2020 (ERN EpiCARE), to support innovative human research.

The main results are: We collected 9523 patients submitted to epilepsy surgery in 36 epilepsy centres across 12 European countries. We comprehensively describe the prevalent spectrum of epileptogenic brain lesions and their topographic brain distribution in 6900 adults and 2623 children, in correlation with key landmarks of underlying medical histories. One year after surgery, 60.7% of patients were seizure free with outcome data available from 7286 patients.

Therefore, the European database represents a benchmark for the diagnosis of surgically amenable brain lesions associated with pharmaco-resistant focal epilepsy (Blümcke et al. NEJM 377:1648-1656, 2017). It also paves the way to successfully study genetic markers for disease classification and to identify molecular targets for personalised treatment. We are suggesting the following way forward, as our results justify a mandate for ongoing research.

We propose to implement large-scale genetic and epigenetic analysis from archival human brain tissues in order to develop a comprehensive, open-access clinico-pathological and genetic database. This will help to also identify non-invasive biomarkers in need to accurately identify epileptogenic brain lesions and to decipher lesion-specific drug targets that can be translated into successful new therapies.

Ingmar Blümcke

Biography
Professor Blümcke is Director of the Dept. of Neuropathology at the University Hospital Erlangen (Germany). His research addresses clinical and molecular neuropathology of human epilepsy brain tissue specimens. Dr. Blümcke founded the European Epilepsy Brain Bank in 2006, which comprises up to date 10,000 epilepsy surgery specimen. Other achievements of his scientific carrier include a first international consensus classification of Focal Cortical Dysplasias (2011) and of Hippocampal Sclerosis (2013). His scientific work is documented by more than 270 peer-reviewed articles with a current h-factor of 58. Dr. Blümcke’s research is continuously funded by the EU-FP6 (LSHM CT-2006-037315), -FP7 (GA#602531) and -H2020 (GA#769051) health programmes.

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DESIRE & EpiCARE

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Anni Ahonen-Bishopp

Biography
Anni Ahonen-Bishopp gained her PhD in the molecular neurobiology group of Prof. Heikki Rauvala, which in 2003 joined the Helsinki Neuroscience Center. During her PhD Anni specialised in cross-disciplinary research projects, acquiring knowledge and skills in biochemistry and physiology. At the end of her PhD she supervised master students and ran practical courses. Anni moved to work with Biocomputing Platforms as a science expert, and later as a team leader. Her main responsibilities are solution design, translational research projects, and science communications. Anni’s position has given her insights to the field of genetics in relation to the practical everyday issues.

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Project
EpimiRNA

Multi-omic atlas of functional microRNA for epilepsy

EpimiRNA central database hosts the research data collections for EpimiRNA consortium, and is used to accumulate knowledge about the micro-RNA (miRNA) targets identified during the project. The central database links with public EpimiRBase database (http://www.epimirbase.eu/) containing curated information on microRNA epilepsy associations from human, mouse and rat samples consisting of over 2000 associations in total.

EpimiRNA central database contains expression, proteomics and miRNA data from animal and human samples. The collection consists of 72 mouse, 168 rat, and 19 human Ago miRNA samples, 19 rat RNA sequencing (RNAseq) samples, 24 rat proteomics samples, and 9 human Ago iCLIP samples. Ago iCLIP methodology in this context is presented in more detail in an associated poster presentation by M. Venø.

The underlying central database structure and software allow this information to be linked with other such knowledge-base instances, in order to create a network for analytical and secure result sharing purposes. The platform provides programmatic access to data, and various statistical tools for in situ analysis and discovery work. When connected to a larger network of datasets, various meta-analysis options can be utilised.

The EpimiRNA central database is hosted by University of Århus and it is available only for consortium members and possible research collaborators. The system consists of BC Platform’s database and analytics solution connected to HPC cluster consisting 196 computing nodes and 3384 cores.
Roland Krause

Biography
Roland Krause is a research associate at the University of Luxembourg’s Luxembourg Centre for Systems Biomedicine. He is specialised in data management and data stewardship for international research collaboration and supports several projects in epilepsy genetics in this role, notably EpiPGX and the ILAE consortium on complex genetics of the epilepsies. He serves as the training coordinator for Elixir Luxembourg. His research on integrating omics data from multiple sources focuses on the elucidation of mechanisms of epilepsies and other neurological syndromes.

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Project
EpiPGX

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Controlled access resources for genetic and pharmacogenetics research for epilepsy

For clinical genetic studies, global collaborations are required to collect statistically relevant numbers. Omics for transcriptomics, proteomics and epigenetic data from many sources are becoming available in increasingly large volumes that can no longer be managed by individual researchers.

The integration of said data provide challenges with respect to data ownership, ethics and data protection but also mundane IT infrastructure.

I will review the current state of collaboration across the epilepsy community and provide a forward-looking view on how data sharing and research collaboration can be driven by the community.
Eleonora Aronica

Biography

Eleonora Aronica is full professor of Neuropathology at the University of Amsterdam's Faculty of Medicine (AMC-UvA). She obtained her doctorate cum laude at the UvA in 1993 after studying medicine at the University of Catania (Italy), where she completed her studies as a neurologist. She leads a research group focused on translational research into Epilepsy and aiming to understand the pathogenesis, epileptogenesis and pharmacoresistance. Her scientific honours include the Michael Prize (2011) for epilepsy research. She has published more than 300 peer-reviewed articles. She is member of the ILAE Task Force on FCD and international European consortia (FP7, Horizon 2020).

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Project

EPISTOP

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Biobanking in Tuberous Sclerosis Complex: challenges and opportunities for understanding epilepsy and cognitive and behavioral co-morbidities

The use of human brain tissue represents a crucial strategy to advance our understanding of the underlying neuropathology and the molecular and cellular basis of epilepsy and related cognitive and behavioural co-morbidities. There is a specific need for well-documented and high quality human brain tissue from severe paediatric epilepsies. In addition to studies on surgical samples, postmortem human brain tissue from subjects with and without epilepsy has become useful for a better understanding of the complex network changes associated with epilepsy and related co-morbidities. Generation of primary and (stem)cell lines from human tissue may further enrich this resource and provide the research community with additional tools to study epilepsy associated cellular abnormalities.

Tuberous Sclerosis Complex (TSC) represents a model disease for investigating mammalian target of rapamycin (mTOR)-related epileptogenesis. TSC molecular networks will be discussed, highlighting evidence for shared cellular and molecular mechanisms underlying non-TSC related epilepsies. Ignoring the value of human tissues in both clinical and basic research can compromise validity and quality of research.

The collection and use of well-documented and high quality human brain tissues from severe paediatric epilepsies requires a multidisciplinary approach embedded in international research networks. The long-term sustainability of tissue collections represents a major challenge. In addition, expert knowledge in neuropathological diagnostic criteria with harmonisation of procedures-protocols, and implementation of common standards for ethical and legal issues requires further streamlining. In conclusion, there is a clear need for long-term investment in order to optimise the potential of biobanking for rare genetic diseases.
Asla Pitkänen

Biography

Asla Pitkänen is Professor of Neurobiology at the University of Eastern Finland. Her neurosciences research at the A.I. Virtanen Institute ranges from the cellular and molecular level to experimental animal models and brain imaging. Her research goal is to identify early pathophysiological mechanisms following traumatic brain injury, which can be used as biomarkers and treatment targets to prevent post-traumatic epileptogenesis. She is involved as a Principal Investigator and Workpackage Leader in the EU-funded research project EPITARGET (www.epitarget.eu) amongst others.

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Project

EPITARGET

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Database for powered preclinical multicenter trial

Issues related to reproducibility of preclinical data and lack of translation of preclinical discoveries to new epilepsy therapies has encouraged researchers to develop new methodologies to increase the power and reproducibility of preclinical studies. EPITARGET tailored common data elements (CDEs) for preclinical experiments performed in the consortium to be used in a shared database and in individual laboratories. CDEs are organized into eight major case report form (CRF) modules, presenting >1000 data points for each animal. EPITARGET CDEs and CRFs present the first effort for harmonization of data collection and analysis in preclinical epilepsy research. The CDEs were further implemented for constructing a data dictionary for a browser-based, metadata-driven database in REDCap. The great challenge will be to develop a sustainable open access European Epilepsy Data Ecosystem that integrates preclinical and clinical databases for biomarker and treatment discovery in epilepsy.
Poster Abstracts

1 Biomarkers
2 Genetics
3 Therapeutics
4 Co-Morbidities
5 Biobanks and Databases
1 Biomarkers

Poster Abstracts
miRNAs are short strands of RNA involved in post-transcriptional regulation of gene expression, and miRNA dysregulation has been implicated in epilepsy pathogenesis. Identifying the functional impact of such dysregulation is far from straightforward, with many studies limited to subjective selection of miRNAs and/or their gene targets. As part of the EpimiRNA consortium, we performed Weighted Gene Co-expression Network Analysis (WGCNA) on miRNA RNA-Seq data from the hippocampi of rats exposed to perforant path stimulation and identified modules of co-regulated miRNAs that are differentially expressed at early and late stages of the epileptogenic phase in this animal model. While some miRNAs were differentially co-regulated throughout epileptogenesis, others were present only at early or late stages, suggesting persistent but varying miRNA-mediated regulation during epileptogenesis and highlighting the potential to discriminate these stages and target therapy accordingly. To investigate the functional role of these modules, we developed an unbiased, systems-level approach to prioritise miRNA-gene target interactions, and identified multiple epilepsy-related genes and pathways. Finally, we correlated miRNA expression to mRNA RNA-Seq data from the same animals to infer a functional miRNA-mRNA network active during epileptogenesis. Together, our approaches can be used to thoroughly characterise miRNA dysregulation and its functional impact in epileptogenesis.

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3 The effect of anti-epileptic drugs and disease modifying treatments on microRNA biomarkers in the intra-amygdala kainic acid model of Temporal Lobe Epilepsy

Abstract

MicroRNAs are small non-coding RNAs that regulate translation of proteins and show potential as biomarkers of epilepsy. To this end, we have identified and validated a diverse group of microRNAs in plasma using 3 animal models of epilepsy. Here for the first time, we test whether currently available anti-epileptic drugs (AEDs) or a disease-modifying treatment (antagomir-134) alters the expression of these biomarkers. The mouse intra-amygdala kainic acid model of epilepsy was used in these studies. Animals with epilepsy were treated with AEDs twice daily (IP) for 3 days and plasma was analysed for changes in microRNA expression. In a separate study, animals were injected with antagomir-134 (ICV) 1 hour after kainic acid injection and plasma was analysed after 2 weeks. The expression of the biomarker microRNAs was not affected by the AEDs, but a number were affected by the disease-modifying treatment. This demonstrates that our biomarkers are sensitive to the underlying disease and not to seizures alone.

Authors

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4 Prenatal MRI and correlation with outcome at two years in children with Tuberous Sclerosis Complex.

Abstract

Purpose: to assess the correlation between prenatal MRI findings and neurodevelopmental outcome and refractory epilepsy at the age of 2 years. Methods: children with definite TSC and follow-up up to two years of age whose prenatal MRI scan was available were included. The MRI scans were scored according to the number of lesions per lobe: 0 = no lesions or doubtful, 1 = small lesion and 2 = ≥ 1 small lesion or ≥ 1 large lesion. Clinical characteristics were related to lesion sum score (low: below mean lesion sum score of cohort; high: ≥ mean). Results: 18 patients were included. Mean gestational age at MRI was 32.7 weeks. TSC lesions were present in 94.4%. The mean sum score of lesions was 4. At 2 years, 38.9% of patients had refractory epilepsy and 50% had intellectual disability. Both refractory epilepsy and intellectual disability were significantly more frequent in patients with a high sum score than patients with a low sum score, respectively 63.3% vs. 0% (p=0.013), and 60% vs. 0% (p=0.026). Conclusion: this study shows that children with TSC and a low lesion sum score on prenatal MRI have a better clinical outcome at two years of age. These findings may assist prenatal counselling in patients with TSC.

Authors

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miRNAs can alter levels in serum or plasma in neurological diseases making them attractive candidates for peripheral biomarkers of epilepsy. This study was conducted to evaluate usefulness of plasma miRNAs as biomarkers of epileptogenesis and epilepsy. We used the rat model of Temporal Lobe Epilepsy induced by status epilepticus evoked by the stimulation of the left lateral nucleus of the amygdala. Blood was collected at 14, 30, 60, and 90 days after stimulation from the tail vein and plasma was processed using Affymetrix miRNA 4.1 arrays. We have compared miRNA levels between sham operated and stimulated animals (p<0.01). We have detected 14 miRNAs differentiating between sham operated and stimulated animals at 14 days, 6 at 30 d, 16 at 60d, and 11 at 90 d. We have also compared miRNA levels between animals with high and low numbers of seizures. We found differences in levels of 11 miRNAs at 14 d, 7 at 30 d, 11 at 60 d, and 8 at 90 d (at p<0.01). Levels of miRNA in plasma are altered during epileptogenesis and differentiate between animals with frequent and rare seizures. miRNA may become a useful biomarker of epileptogenesis/epilepsy as well as severity of the disease.

Authors
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1) Laboratory of Epileptogenesis, Nencki Institute of Experimental Biology, Warsaw, Poland.

Abstract
Circulating miRNA as a biomarker of epileptogenesis and in the rat model of Temporal Lobe Epilepsy

Tuberous Sclerosis Complex (TSC) is frequently associated with severe intractable epilepsy. In some TSC patients epilepsy surgery is a promising treatment option provided that the epileptogenic zone can be precisely delineated. TSC cortical tubers contain dysmorphic neurons, brightly eosinophilic giant cells and white matter alterations. However, a histological classification system has not been established for tubers. Therefore, the aim of this study was to define distinct histological patterns within tubers based on clinicopathological and histopathological correlations. In total, we studied 28 cortical tubers and seven samples of perituberal cortex from 28 TSC patients. We assessed the mammalian target of rapamycin complex 1 (mTORC1) activation, the numbers of giant cells, dysmorphic neurons, neurons, and oligodendrocytes, as well as and calcification, gliosis, angiogenesis, inflammation, and myelin content. Three distinct histological profiles emerged based on the proportion of calcifications, dysmorphic neurons and giant cells designated types A, B, and C. In the latter two types we were able to associate them with features on presurgical MRI. Therefore, these histopathological patterns provide consistent criteria for improved definition of the clinico-pathological features of cortical tubers identified by MRI and provide a basis for further exploration of the functional and molecular features of cortical tubers.

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6 Novel histopathological patterns in cortical tubers of epilepsy surgery patients with Tuberous Sclerosis Complex

Abstract
Tuberous Sclerosis Complex (TSC) is frequently associated with severe intractable epilepsy. In some TSC patients epilepsy surgery is a promising treatment option provided that the epileptogenic zone can be precisely delineated. TSC cortical tubers contain dysmorphic neurons, brightly eosinophilic giant cells and white matter alterations. However, a histological classification system has not been established for tubers. Therefore, the aim of this study was to define distinct histological patterns within tubers based on clinicopathological and histopathological correlations. In total, we studied 28 cortical tubers and seven samples of perituberal cortex from 28 TSC patients. We assessed the mammalian target of rapamycin complex 1 (mTORC1) activation, the numbers of giant cells, dysmorphic neurons, neurons, and oligodendrocytes, as well as and calcification, gliosis, angiogenesis, inflammation, and myelin content. Three distinct histological profiles emerged based on the proportion of calcifications, dysmorphic neurons and giant cells designated types A, B, and C. In the latter two types we were able to associate them with features on presurgical MRI. Therefore, these histopathological patterns provide consistent criteria for improved definition of the clinico-pathological features of cortical tubers identified by MRI and provide a basis for further exploration of the functional and molecular features of cortical tubers.

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**7 EEG as a biomarker in experimental epileptogenesis**

**Abstract**

Identification of EEG patterns which are specifically present or absent during epileptogenesis (EPG) would possibly allow preventive treatment. Methods: In rats, EPG was induced by repeated electrical stimulation of the perforant pathway. Baseline (BL), EPG and manifest epilepsy (MFE) EEG were recorded from the dentate gyrus. For EEG epochs, 7 features and the spectral power in different frequency bands were calculated. Results: Only 1 feature (Asymmetry) showed consistent changes over time. Power in delta and theta bands decreased, while beta and gamma increased during EPG and MFE. A neural net, trained to detect epochs of epileptogenesis based on 1 s of EEG with 7 features, achieved a positive predictive value (PPV) of 61%. Conclusion: Although we identified EEG features that differed between BL and EPG, our method did not yet reach a sufficient PPV. Possible enhancements of machine learning methods will include modified input data and additional features.

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**8 Biomarkers of epileptogenesis: the focus on glia and cognitive dysfunctions**

**Abstract**

We explored minimally invasive means to reliably predict the onset of epilepsy after brain injury in animal models to discover biomarkers that could increase the sensitivity of the existing clinical indicators. Our approaches include molecular brain imaging and the measure of soluble molecules in blood which may reflect brain intrinsic events involved in epilepsy development. Among these, we focused on 1H-MRS-based analysis of astrocyte activation, and the blood levels of the astrocyte-related protein HMGB1, since this cell population appears to be pivotal in epileptogenesis triggered by differing brain insults. Moreover, we also investigated behavioral biomarkers by focusing on cognitive dysfunctions since this deficit represents a typical co-morbidity in epilepsy which may manifest even before the onset of spontaneous seizures. Our evidence demonstrate the prognostic value of measuring myo-inositol in the hippocampus by MRS as a marker of astrocyte’s activation, and the associated cognitive deficits, which allowed early stratification of animals developing epilepsy. Blood HMGB1 levels also discriminated with high fidelity between animals developing or not developing spontaneous seizures early after brain injury. Our findings can be easily translated to the clinical setting for enriching the patient population in preventive clinical trials designed to study anti-epileptogenic treatments.

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Temporal Lobe Epilepsy (TLE), the most common form of focal epilepsy, is associated with large-scale molecular and cellular changes during epileptogenesis. Currently, one-third of all TLE patients are pharmacoresistant due to incomplete understanding of the molecular mechanisms of the disease. The long-standing neuron-centric view of TLE is changing as the role of glial cells is being explored. Recent studies have shown that non-coding RNAs, like microRNAs, act at a post-transcriptional level in neural cells and contribute to the pathogenesis and progression of TLE. MicroRNAs are small non-coding RNAs (~22 nucleotides) that regulate messenger RNA expression by binding to their 3' untranslated regions. Genome-wide miRNA profiling studies performed on both human TLE tissue and from animal models revealed significant changes in miRNA expression levels in diseased condition compared to controls. Using an intra-cortical kainate mouse model, glial cells will be isolated and sequenced for miRNAs. In-vitro and in-vivo validations will be done to establish the functional contribution of these miRNA with respect to glial cells in TLE. Furthermore, techniques to manipulate the miRNA expression levels will be performed to assess rescue of seizure phenotype. Our study will help grasp the bigger picture of TLE progression and explore miRNAs as effective TLE therapeutics.

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Understanding glial miRNAs in the pathogenesis of Temporal Lobe Epilepsy

Abstract

Temporal Lobe Epilepsy (TLE), the most common form of focal epilepsy, is associated with large-scale molecular and cellular changes during epileptogenesis. Currently one-third of all TLE patients are pharmacoresistant due to incomplete understanding of the molecular mechanisms of the disease. The long-standing neuron-centric view of TLE is changing as the role of glial cells is being explored. Recent studies have shown that non-coding RNAs, like microRNAs, act at a post-transcriptional level in neural cells and contribute to the pathogenesis and progression of TLE. MicroRNAs are small non-coding RNAs (~22 nucleotides) that regulate messenger RNA expression by binding to their 3' untranslated regions. Genome-wide miRNA profiling studies performed on both human TLE tissue and from animal models revealed significant changes in miRNA expression levels in diseased condition compared to controls. Using an intra-cortical kainate mouse model, glial cells will be isolated and sequenced for miRNAs. In-vitro and in-vivo validations will be done to establish the functional contribution of these miRNA with respect to glial cells in TLE. Furthermore, techniques to manipulate the miRNA expression levels will be performed to assess rescue of seizure phenotype. Our study will help grasp the bigger picture of TLE progression and explore miRNAs as effective TLE therapeutics.
2 Genetics

Poster Abstracts
Subependymal giant cell astrocytomas in tuberous sclerosis complex have consistent TSC1/TSC2 biallelic inactivation, and no BRAF mutations

Abstract

Biallelic alterations in TSC2, and less commonly, TSC1, are consistently seen in SEGAs. Approximately 10% of SEGAs have mutations in TSC1, while 5 had no mutation identified in TSC1/TSC2. The majority of these samples had loss of heterozygosity in the same gene in which the mutation was identified. These results significantly extend previous studies, and in agreement with the Knudson two hit mechanism indicating that these alterations in TSC2 and TSC1 are not only rare, but also common.

Subependymal giant cell astrocytomas (SEGAs) are the most common and frequently observed brain tumours associated with tuberous sclerosis complex (TSC). SEGAs are low-grade neoplasms and arise from abnormal astrocytes that develop in the lateral ventricles. The pathogenesis of SEGAs in TSC is thought to involve two-hit inactivation of TSC1 or TSC2. Genetic alterations, such as frameshift and nonsense mutations in TSC1 or TSC2, are observed in SEGAs. The current study aimed to determine the prevalence of genetic alterations in SEGAs and their relationship to the clinical and histological features of the tumours.

Studies have shown that SEGAs in TSC patients often have mutations in TSC1 or TSC2, and that these mutations are often associated with loss of heterozygosity (LOH) in the same gene. The current study aimed to determine the prevalence of genetic alterations in SEGAs and their relationship to the clinical and histological features of the tumours.

Methods

Samples were collected from patients with TSC and SEGAs. DNA was extracted from formalin-fixed, paraffin-embedded tissue, and DNA-seq analysis was performed using a targeted genome-wide panel of 200 genes. The results were compared with clinical and histological data.

Results

Approximately 10% of SEGAs had mutations in TSC1, while 5 had no mutation identified in TSC1/TSC2. The majority of these samples had loss of heterozygosity in the same gene in which the mutation was identified. These results significantly extend previous studies, and in agreement with the Knudson two hit mechanism indicating that these alterations in TSC2 and TSC1 are not only rare, but also common.
12 De novo mutations of the ATP6V1A gene cause developmental encephalopathy with epilepsy

Abstract
V-type proton (H+) ATPase (v-ATPase) regulates pH homeostasis. In neurons, it plays additional and unique roles in synapse function. Through WES, we identified de novo heterozygous mutations (p. P27A, p.D100T, p.D349N, and p.D371G) in the A subunit of v-ATPase (ATP6V1A) in four patients with developmental encephalopathy with epilepsy. Functional studies in HEK293T cells and patients' lymphoblasts demonstrated gain of function for p.D349N (increased proton pumping in intracellular organelles), and loss of function for p.D100T (decreased expression of ATP6V1A and reduced levels of lysosomal markers). In rat hippocampal neurons, p.D349N and p.D100T caused a similar defect in neurite elongation and loss of excitatory inputs. This study provides evidence that de novo heterozygous ATP6V1A mutations cause a developmental encephalopathy with perturbations of lysosomal homeostasis and neuronal connectivity. Genotype/phenotype correlation in 16 newly identified patients carrying mutations in ATP6V1A is currently ongoing.

Authors
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13 A novel double-hit mechanism involving different genes of the MTOR pathway in hemimegalencephaly with intractable epilepsy

Abstract
Through a NGS study in patients with epilepsy, intellectual disability, and malformations of cortical development, we identified a patient with hemimegalencephaly (HME) and intractable epilepsy carrying mosaic mutations in two MTOR pathway genes (MTOR and RPS6). The mutation in MTOR (p.S2215F, 5% of mosaicism in dysplastic brain tissue only) had previously been related to focal cortical dysplasia and HME. The mutation in RPS6 (p.R232H, 11% of mosaicism in blood, 14% in dysplastic brain) is a novel finding. Expressing the mutations in cellular and animal models, we demonstrated that both caused MTOR pathway hyperactivation and increased mutant cells size. p.S2215F also caused a neuronal migration delay, while p.R232H an increased cell proliferation. Here, we show that, in addition to single activating mutations and ‘double hit’ inactivating mutations in MTOR pathway genes, HME can result from activating mutations affecting different genes of this pathway. Our data also indicate RPS6 as a novel disease-related gene.

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V-type proton (H+) ATPase (v-ATPase) regulates pH homeostasis. In neurons, it plays additional and unique roles in synapse function. Through WES, we identified de novo heterozygous mutations (p. P27A, p.D100T, p.D349N, and p.D371G) in the A subunit of v-ATPase (ATP6V1A) in four patients with developmental encephalopathy with epilepsy. Functional studies in HEK293T cells and patients' lymphoblasts demonstrated gain of function for p.D349N (increased proton pumping in intracellular organelles), and loss of function for p.D100T (decreased expression of ATP6V1A and reduced levels of lysosomal markers). In rat hippocampal neurons, p.D349N and p.D100T caused a similar defect in neurite elongation and loss of excitatory inputs. This study provides evidence that de novo heterozygous ATP6V1A mutations cause a developmental encephalopathy with perturbations of lysosomal homeostasis and neuronal connectivity. Genotype/phenotype correlation in 16 newly identified patients carrying mutations in ATP6V1A is currently ongoing.

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Several features of Dravet syndrome (DS) patients are replicated in heterozygous Nav1.1 knockout mice. Published results obtained with these mice have shown selective reduction of sodium current and excitability in GABAergic interneurons, reduced inhibition and hyperexcitability in cortical and hippocampal circuits, also in the pre-epileptic period. Surprisingly, no modifications in neocortical dynamics have been observed in vivo in the cortex at physiologic temperature (DeStasi et al 2016 Cerebral cortex 26:1778), consistent with the presence of homeostatic responses. We have performed patch-clamp experiments in hippocampal slices of Nav1.1+/- KO mice in the pre-epileptic period (PN14-18) for disclosing homeostatic responses. We investigated the properties of different subtypes of interneurons involved in inhibitory microcircuits. Our results show an impairment of firing and a reduction of the slope of the action potentials in some interneurons, (e.g. parvalbumin positive basket-cells), but an increased excitability in other subtypes. We show that this is a homeostatic response in DS mice able to ameliorate the phenotype of the mouse model. Interestingly, this response may possibly be at play also in other pathologies characterized by hypoexcitability of GABAergic neurons.

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Tuberous sclerosis complex (TSC) is a rare, genetic disease caused by loss-of-function mutations in either TSC1 or TSC2. In most patients, the CNS is affected due to the presence of structural abnormalities in the brain. Functional CNS abnormalities consist of epilepsy and TSC-associated neuropsychiatric disorders. Epilepsy is the most common presenting symptom of TSC, as it affects up to 90% of TSC patients. In this research, we investigated the brain phenotype of TSC using the tsc2vu242 zebrafish model. The homozygous (tsc2-/-) larvae displayed enlarged brains, reduced locomotor behaviour and epileptiform discharges at 7 dpf. Rapamycin, a well-known mTOR inhibitor, was shown to have a significant rescue effect on selected phenotypic readouts as well as at the molecular level. These results demonstrate that the tsc2-/- zebrafish model mirrors certain aspects of the human condition.

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3 Therapeutics

Poster Abstracts
Matrix metalloproteinases (MMPs) play a key role in the remodelling of the extracellular matrix and can contribute to epileptogenesis. Currently, there are no selective MMP inhibitors, and non-selective inhibitors have strong side effects and toxicity, limiting their therapeutic use. We therefore investigated MMP expression in the hippocampus of patients with Temporal Lobe Epilepsy (TLE) and in the rat TLE model. Furthermore, the effects of the broad-spectrum MMP inhibitor minocycline and the recently developed MMP9 inhibitor IPR-179 were studied in epileptogenesis using the rapid kindling model. Increased expression of MMP2, 3, 9 and 14 was observed in the hippocampus of TLE patients and throughout epileptogenesis in the rat hippocampus. The novel MMP9 inhibitor IPR-179, but not the broad-spectrum inhibitor minocycline, had anti-ictogenic and anti-epileptogenic effects in the rapid kindling model. Therefore, IPR-179 deserves further investigation as a novel therapeutic agent. This research was supported by the Dutch Epilepsy Foundation, project number EF 16-05, the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no 602102 (EPITARGET) and the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no 642881 (ECMED).

17 Attenuation of epileptogenesis in the rat by IPR-179, a novel matrix metalloproteinase-9 inhibitor

Abstract
Matrix metalloproteinases (MMPs) play a key role in the remodelling of the extracellular matrix and can contribute to epileptogenesis. Currently, there are no selective MMP inhibitors, and non-selective inhibitors have strong side effects and toxicity, limiting their therapeutic use. We therefore investigated MMP expression in the hippocampus of patients with Temporal Lobe Epilepsy (TLE) and in the rat TLE model. Furthermore, the effects of the broad-spectrum MMP inhibitor minocycline and the recently developed MMP9 inhibitor IPR-179 were studied in epileptogenesis using the rapid kindling model. Increased expression of MMP2, 3, 9 and 14 was observed in the hippocampus of TLE patients and throughout epileptogenesis in the rat hippocampus. The novel MMP9 inhibitor IPR-179, but not the broad-spectrum inhibitor minocycline, had anti-ictogenic and anti-epileptogenic effects in the rapid kindling model. Therefore, IPR-179 deserves further investigation as a novel therapeutic agent. This research was supported by the Dutch Epilepsy Foundation, project number EF 16-05, the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no 602102 (EPITARGET) and the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no 642881 (ECMED).

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18 Target site-specific effects of brain stimulation on microRNA expression in experimental epilepsy

Abstract
Rationale: Deep brain stimulation (DBS) is an approved treatment for mesial Temporal Lobe Epilepsy (mTLE). Mechanisms of action are mostly unknown. Methods: MTLE was induced by repetitive electrical stimulation of the perforant pathway (PP) in rats. We investigated the effect of DBS of the PP on the formation of the dentate gyrus and the ventral hippocampal commissure (VHC). We applied both high frequency and low frequency stimulation. Furthermore, we investigated microRNA regulation in the hippocampus after DBS with the most effective paradigm by next generation sequencing. Results: Low frequency DBS of the VHC significantly prolonged the latency period. 8 microRNAs were significantly altered directly after VHC stimulation. Upregulation of 5 microRNAs measured after a 97-day recording period was suppressed by VHC-stimulation. Conclusion: MicroRNAs whose upregulation is prevented by low frequency VHC-stimulation are candidates for an in vivo manipulation to inhibit development of epilepsy.

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Epilepsy is a brain disease that affects over 50 million people worldwide. Evidence emerged that miRNAs play an important role in epilepsy. MiR-22 regulates key epileptogenic-related processes, such as inflammation and apoptosis. Our group has detected increased levels of miR-22 in non-necrotic brain regions after status epilepticus (SE). Here, we sought genetic evidence that miR-22 affects epileptogenesis and epilepsy. Firstly, we characterized naïve miR-22 knockout and compared to wild-type. No differences were observed in the brain morphology, gliosis or astrocytosis. We also found that other microRNA have not been affected by the ablation of miR-22. When injected with KA intra-amygdala mice lacking miR-22 presented similar seizure severity when compared to wild-type mice and similar neuronal damage in the hippocampus. Interestingly, miR-22 knockout mice presented increased number of spontaneous seizures, when monitored for 2 weeks. RNA sequencing revealed that 24h after SE miR-22 mice presented an increased expression in genes related to inflammatory response and apoptosis. Here, we also verified that osmotic mini pumps can be used to continuous deliver miR-22 mimics into the brain. These data indicate that miR-22 plays a role in the pathophysiology of epilepsy, and that targeting this miRNA may be a promising approach for epilepsy treatment.

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Epilepsy is a devastating neurological disorder affecting over 50 million people worldwide, with no effective cure to date. New protein-based drugs with anti-epileptic potential are being investigated. However, their poor brain penetration upon systemic administration due to the blood-brain barrier (BBB) leads to low efficiency and side effects, hence posing a substantial challenge. Improving their bioavailability in the brain would open a new promising avenue towards the treatment of epilepsy and potentially other disorders affecting the central nervous system. We developed the Molecular Envelope Technology (MET), which essentially comprise biocompatible polymeric nanoparticles that can enhance the transfer of hard-to-deliver drugs across biological barriers. Using this proprietary nanotechnology, we engineered a hybrid protein-polymer system with favourable physicochemical characteristics that appears to facilitate brain delivery and increases the levels of the therapeutic protein cargo in neural tissue following intranasal or subcutaneous administration, as shown by our pharmacokinetics studies in rodents. We next aim to explore the therapeutic effects of our formulation in animal models of epilepsy. If proven effective, it could potentially improve clinical outcome in patients suffering from epilepsy and reduce associated side effects.

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Enhanced brain delivery of a potentially anti-epileptic drug using Molecular Envelope Technology (MET)

Abstract
Epilepsy is a devastating neurological disorder affecting over 50 million people worldwide, with no effective cure to date. New protein-based drugs with anti-epileptic potential are being investigated. However, their poor brain penetration upon systemic administration due to the blood-brain barrier (BBB) leads to low efficiency and side effects, hence posing a substantial challenge. Improving their bioavailability in the brain would open a new promising avenue towards the treatment of epilepsy and potentially other disorders affecting the central nervous system. We developed the Molecular Envelope Technology (MET), which essentially comprise biocompatible polymeric nanoparticles that can enhance the transfer of hard-to-deliver drugs across biological barriers. Using this proprietary nanotechnology, we engineered a hybrid protein-polymer system with favourable physicochemical characteristics that appears to facilitate brain delivery and increases the levels of the therapeutic protein cargo in neural tissue following intranasal or subcutaneous administration, as shown by our pharmacokinetics studies in rodents. We next aim to explore the therapeutic effects of our formulation in animal models of epilepsy. If proven effective, it could potentially improve clinical outcome in patients suffering from epilepsy and reduce associated side effects.

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22 A novel exosome-like nanocarrier for treatment of refractory epilepsy

Abstract
Treatment of brain diseases is a tremendously difficult task due to the hindered passage of therapeutics through the blood-brain barrier (BBB). The highly selective properties of the BBB result in 1/3 of the 50 million epileptics worldwide being refractory to treatment. Providing a novel treatment for refractory epilepsy would be of great international value, as it would alleviate reduced life quality and decrease socio-economic costs. Here, we will enable novel treatment of refractory epilepsy by exploiting the properties of endogenous exosomes to produce an exosome-like nanocarrier for safe, targeted delivery of antiepileptic drugs across the BBB. Exosomes are small membrane-bound organelles that can transmit macromolecules from one cell to a specific target cell. Exosomes can diffuse across the BBB and are very stable, making them an ideal candidate in the development of drug delivery systems for brain diseases. Clinical application of exosomes is currently restricted due to limited control of the antigenic response they may cause. Our exosome-like nanocarrier will be developed to efficiently cross the BBB without triggering harmful cellular responses. Our nanocarrier will not only benefit refractive epileptics, but holds significant potential for future adaptations of the nanocarrier in the treatment of other major brain diseases.

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A novel exosome-like nanocarrier for treatment of refractory epilepsy
The epimiRNA consortium has identified antagomirs targeting miRs as promising novel therapeutics for epilepsy. These oligonucleotides exhibit potent anti-epileptic effects in multiple disease models, but their mechanism of action is unclear. We used ex vivo brain slices to explore the effects of candidate antagomirs on hippocampal circuitry.

Antagomirs were administered to adult male Sprague Dawley rats via intracerebroventricular injection. Rats completed a novel object location (NOL) test the following day and acute hippocampal slices were prepared 2-4 days post injection. Single cell properties were measured with patch clamp recording. Network excitability was probed using electrical stimulation and, separately, by acute seizure challenge with 4-aminopyridine. Synaptic plasticity was tested with paired-pulse facilitation and LTP protocols. One slice from each animal was stained for PSD-95 and VGLUT1. Slices from all antagomir treated groups showed reduced excitability in response to electrical stimulation, whilst plasticity was largely unaffected. We did not observe obvious effects on pyramidal cell biophysics, response to seizure challenge or NOL test performance. One candidate appeared to be associated with reduced post-synaptic spine size. All antagomirs reduced hippocampal excitability, which can explain their anti-seizure effect. It is likely that this general change is mediated by different specific mechanisms for each antagomir.
Common causes for refractory epilepsies comprise developmental malformations of the cortex, such as gangliogliomas (GG) and focal cortical dysplasia type IIb (FCDIIb). Both epileptogenic lesions share the disruption of dendritic architecture in dysplastic neurons as a hallmark. Recently, reduced expression levels of the Ste20-like kinase (SLK) were found in dysplastic neurons of human GG and FCDIIb sections. Experimental knockdown of SLK in embryonic mouse brains impaired dendritic arborization and reduced the number of inhibitory synapses in neurons. First experiments to elucidate underlying mechanisms pointed to the inhibitory postsynaptic protein gephyrin as a possible substrate of SLK. Identifying interaction partners of SLK will shed light on SLK’s function in neuronal development and in the formation of dysplastic neurons. Taken together, impaired maintenance of inhibitory synapses emerges as a novel pathogenetic aspect of epileptogenic malformations and requires consideration for therapy development.

Abstract

Epilepsy-associated malformations reveal dysregulation of inhibitory synaptic connections and distal neuronal complexity by the Ste20-like kinase

Neuroinflammation and oxidative stress occur in epileptogenic foci in epilepsy patients and related animal models and their inhibition reduces spontaneous seizures in rodents. We studied whether a combination of drugs blocking the ictogenic IL-1beta/HMGB1 inflammatory signals inhibit epileptogenesis in models of acquired epilepsy. Status epilepticus (SE) was induced in adult male rats by electrical stimulation of the hippocampus or by intra-amygdala injection of kainate in adult male mice. The following combinations of anti-inflammatory or antioxidant drugs, or their vehicles, were injected either in rats (i,ii) or mice (iii) after SE and for the following 1-2 weeks: (i) anakinra and BoxA; (ii) N-acetyl-cysteine and sulforaphane; (iii) VX-765 and Cyanobacterial LPS or the synthetic miR-146a mimic. EEG was recorded from SE onset until the end of treatment as well as in the chronic epilepsy phase. Animals were behaviourally tested for cognitive performance then sacrificed for brain histology. Common therapeutic outcomes were attained after the different drug combinations: the progression in seizure frequency during disease development was abrogated and the frequency of chronic seizures was reduced by 70-90%. Treated animals showed reduced forebrain neurodegeneration and improved memory deficits. Anti-inflammatory and antioxidant drugs should be considered for attaining disease-modifying effects in epilepsy.

Abstract

Combinatorial treatments against neuroinflammation and oxidative stress significantly improved disease outcomes in epilepsy models

Neuroinflammation and oxidative stress occur in epileptogenic foci in epilepsy patients and related animal models and their inhibition reduces spontaneous seizures in rodents. We studied whether a combination of drugs blocking the ictogenic IL-1beta/HMGB1 inflammatory signals inhibit epileptogenesis in models of acquired epilepsy.

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Abstract
Acquired epilepsy is associated with large-scale changes in gene expression, which underlie the cell and network-level changes during epileptogenesis. MicroRNAs play important roles in controlling processes that are dysregulated in epileptogenesis and have emerged as potential therapeutic targets. A key part of the European project EpimiRNA is identifying miRNAs commonly regulated between different animal models of epilepsy. Having recently identified groups of miRNAs common-to-three models during acute, latent and chronic stages of epilepsy we initiated a systematic screen of antimiRs targeting these miRNAs, looking for seizure phenotypes in the intra-amygdala (i.a.) kainic acid (KA) model of status epilepticus in mice. We also explored whether a triple antimiR combo would offer superior protection compared to any single antimiR. Briefly, mice received an antimiR injection (i.c.v.) on the day before i.a. KA. Mice were equipped with EEG and status epilepticus severity was recorded. Brains were analyzed for seizure and inflammation, such as epilepsy. Patients with tuberous sclerosis complex or Temporal Lobe Epilepsy with hippocampal sclerosis. Due to their anti-inflammatory effects, ability to restore aberrant astrocytic neuronal differentiation of human neural stem cells. Similarly to previous evidence for miR146a, miR147b expression was increased in astrocytes in brain tissue from inflammatory response. As previously reported for miR146a, overexpression of miR147b reduced the expression of the pro-inflammatory mediators IL-6 and COX-2 after IL-1 stimulation in both astrocyte and tuberous sclerosis complex cell cultures. miR146a and miR147b overexpression decreased proliferation of astrocytes and promoted inflammatory response. As previously reported for miR146a, overexpression of miR147b reduced the expression of the pro-inflammatory mediators IL-6 and COX-2 after IL-1 stimulation in both astrocyte and tuberous sclerosis complex cell cultures. miR146a and miR147b overexpression decreased proliferation of astrocytes and promoted neuronal differentiation of human neural stem cells. Similarly to previous evidence for miR146a, miR147b expression was increased in astrocytes in brain tissue from patients with tuberous sclerosis complex or Temporal Lobe Epilepsy with hippocampal sclerosis. Due to their anti-inflammatory effects, ability to restore aberrant astrocytic neuronal differentiation and promote neuronal differentiation, miR146a and miR147b deserves further investigation as potential therapeutic targets in neurological disorders associated with inflammation, such as epilepsy.

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A combination of levetiracetam and topiramate exerts disease-modifying effects in the intrahippocampal kainate mouse model

Antiepileptogenesis is a major unmet clinical need in patients at risk. To date, preclinical and clinical trials failed to identify epilepsy-preventing drugs. Considering the multiple mechanisms involved in epileptogenesis, multitargeted approaches were proposed (“network pharmacology”) as an antiepileptogenic treatment strategy. Preclinical development of novel combinations of clinically approved drugs is an innovative approach to generate antiepileptogenic therapies for rapid translation into the clinic. The antiepileptogenic efficacy of the multitargeted drug combination levetiracetam + topiramate was assessed in the intrahippocampal kainate mouse model of epilepsy. After inducing status epilepticus (SE), mice were treated with the drug combination over 5 days t.i.d. Four and 12 weeks post-SE, continuous (24/7) EEG/video-monitoring was performed over 7 days, and electrographic and electroclinical seizures were analysed. In an additional experiment, μMRI and μPET-scanning were used to determine the antiepileptogenic mechanism of the treatment. The multitargeted drug combination levetiracetam + topiramate reduced the number of clinical seizures after drug washout, which was not observed after monotherapy with either drug. The data demonstrate that testing multitargeted drug combinations in this model is a promising approach to face the urgent clinical need for generating antiepileptogenic therapies.

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Understanding the role of miRNA-135a deregulation in Temporal Lobe Epilepsy

The pathogenic mechanisms underlying TLE involve defects in post-transcriptional regulation of gene expression. MicroRNAs (miRNAs) are small (18-25nt long) non-coding RNAs that can modulate the complex gene expression networks which govern the process of epileptogenesis. Previously, we have shown that a significant number of miRNAs are up- or down-regulated in hippocampal tissue of human mTLE patients. One of those miRNAs, miR-135a is upregulated (PMID: 22535415), which can stimulate axon growth and cortical neuronal migration (PMID: 29196317). In the present study, we are investigating the potential role of miR-135a in pathogenesis of TLE via modulating neuronal morphology. We found increased miR-135a levels in intra-amygdala kainate (IAK) mice, and silencing miR-135a expression using antagomirs protected mice from spontaneous epileptic seizures. Further, we identified MEF2 as a potential target that can mediate miR-135a function in TLE. MEF2 proteins are a family of transcription factors which mediate activity-dependent synaptic development (PMID: 19109909). The expression of MEF2 is downregulated in IAK mice and in human mTLE patients with hippocampal sclerosis where the expression of miR-135a is upregulated. Loss of MEF2 in mTLE could lead to abnormal spine formation and contribute to the aberrant firing pattern and cell death observed in epilepsy.

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Epilepsy prevention in patients at risk is a major unmet clinical need. Due to the multiple mechanisms involved in epileptogenesis, the development of multitargeted drug combinations (“network pharmacology”), using clinically approved drugs for rapid translation into clinical trials, was proposed. The drug combination levetiracetam + atorvastatin + ceftriaxone was tested for tolerability and antiepileptogenic efficacy in the intrahippocampal kainate mouse model using an algorithm based on the drug development phases in humans (Klee et al. (2015), Epilepsy Res., 118: 34-48). After confirming the tolerability of the drug combination in naïve and post-SE mice, the drug combination was evaluated for antiepileptogenic efficacy in the intrahippocampal kainate mouse model. We induced a status epilepticus (SE) in groups of male NMRI-mice, which were treated 3 times daily with the drug combination over 5 days during the latent period. Continuous (24/7) EEG- and video-monitoring was performed over 7 subsequent days and 12 weeks post-SE and electrographic and electroclinical seizures were analysed. The analysis of electroclinical seizures indicates no antiepileptogenic effect of the drug combination so far. Further analysis of electrographic seizures and histology will be presented at the meeting.

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32 Activation of the Nrf-2 signaling pathway in epilepsy and its modulation via miRNA-155 in human fetal astrocytes

Abstract
Oxidative stress (OS), a perturbation of the cellular redox state, is evident in the human epileptogenic brain and can therefore present a novel therapeutic target. Here, microRNAs (miRNAs) might pose a therapeutic approach. We investigated OS markers in epileptogenic malformations of cortical development using immunohistochemistry (IHC). Additionally, we stimulated human fetal astrocytes in vitro with H2O2 and analyzed OS markers and miRNAs using IHC, qRT-PCR and Western blot. IHC of epileptogenic brain tissue revealed OS and activation of Nrf-2, an anti-oxidant transcription factor (TF). In vitro, we identified activation of Nrf-2 dependent antioxidant genes and miR-155 to be decreased. MiR-155 transfection boosted oxidant defense and also acted as an anti-inflammatory via suppression of NF-kB signaling. Therefore, we propose that an increase of miR-155 may contribute to limit OS and may be used as a novel therapeutic approach.

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4 Co-Morbidities

Poster Abstracts
Co-morbidities represent additional clinical burden for epilepsy patients. Whilst genetic overlap between neurological disorders (NDs) and epilepsy has been explored, the impact of comorbid NDs to genetic analyses and treatment solutions in epilepsy remains elusive. We tested in a sample of 3934 epilepsy patients, with known pharmacotherapy response status, the hypothesis that genetic predispositions for NDs, represented by polygenic risk scores (PRS), will not be randomly distributed, so that groups of patients can be identified with similar and different patterns of PRS. The main results are as follows: K-means clustering of normalized PRS for schizophrenia, Alzheimer’s, depression, attention-deficit hyperactivity disorder, and migraine identified three distinct cluster of patients. One of the cluster, representing patients with higher genetic predispositions for schizophrenia and Alzheimer’s, emerged as enriched with patients who did not respond to pharmacotherapy (Fisher’s-P=0.002; OR=1.27). The impact of this can be described as follows: Genetic predispositions for NDs have the potential to predict each epilepsy patient’s response to pharmacotherapy. Therefore we can conclude that confirmatory analyses combined with PRS derived from more powered ND-meta-analyses are needed in order to confirm the results, and to explore the aetiology a deeper structure beyond the three identified clusters of patients with a distinct genetic risk makeup.

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Early diagnosis of Tuberous Sclerosis Complex – a chance to prevent epilepsy? How to make the diagnosis before seizures?

Abstract
Tuberous sclerosis complex (TSC) is a genetic disorder highly associated with epilepsy affecting 70 – 90% of patients with the onset usually between 4th - 6th months of life and highly associated with intellectual disability. As recent European recommendations suggest early Vigabatrin treatment if ictal discharges occur on EEG recordings, despite clinical manifestation, we aimed to define the most useful approach to make the diagnosis of TSC before seizure onset (before age 4 months), in order to start early EEG monitoring with possible preventative treatment intervention. Methods: We performed a retrospective review of children who were suspected of having TSC due to single or multiple cardiac tumours as the first sign of the disease. Results: 82/100 children were diagnosed with TSC within the first 4 months of life. Apart from cardiac tumours, the most frequently observed early TSC signs were subependymal nodules (71%), cortical dysplasia (66%), and hypomelanotic macules (35%). Conclusions: Early diagnosis of TSC, before seizure onset, is feasible and becoming pivotal for epilepsy management and improvement of cognitive outcome. Brain MRI, echocardiography, skin examination and genetic testing should be performed early in every patient suspected of TSC.

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5 Biobanks and Databases

Poster Abstracts
35 Common data elements and database for preclinical epilepsy studies

Abstract
Lack of translation of preclinical findings to the clinic has encouraged researchers to develop new methodologies to increase the power and reproducibility of preclinical studies. One approach to facilitate harmonization of data collection and analysis between studies is to develop common data elements (CDEs) and use shared databases. This method has been applied for a long time in clinical studies and EPITARGET has recently followed the example. As the first step of harmonization of procedures between laboratories, working groups were established to design project-tailored CDEs and case report forms (CRFs) for preclinical epilepsy studies. CDEs were organized into eight major CRF modules, presenting >1000 data points for each animal. These CDEs and CRFs present the first single-project effort for harmonization of data collection and analysis in preclinical epilepsy research. The CDEs were further implemented for constructing a data dictionary for a browser-based, metadata-driven database in REDCap.

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36 Genome wide microRNA target site detection in human epileptic brain by Ago iCLIP

Abstract
Several microRNAs (miRNAs) are known to play a role in epilepsy. This makes aberrantly expressed miRNAs, or their direct targets, prime targets for disease modifying epilepsy treatment. However, computational predictions of miRNA targeting are still lacking in precision, suffering from excessive false positives and false negatives. We have utilized Argonaute (Ago) Individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) to generate genome wide maps of direct binding between miRNA and Ago, the miRNA effector protein. Ago-iCLIP was performed on resected hippocampal tissue from mesial Temporal Lobe Epilepsy (mTLE) patients, yielding experimentally validated miRNA binding sites. A database of genome wide miRNA targeting of all expressed miRNAs in mTLE patient hippocampal tissue was generated. This database is a valuable resource of validated miRNA targets for use with our recent miRNA profiling studies in rodents and human resected hippocampus and for miRNA-related studies in general.

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**DESIRe**
Focuses on Epilepogenetic Developmental Disorders (EDD), i.e. early onset epilepsies whose origin is closely related to developmental brain processes. The project studies genetic and epigenetic factors and develops novel diagnostics and treatment strategies.

**EpiCARE**
Is a European Reference Network focusing on rare and complex epilepsies, aiming to develop and deliver highly-specialised diagnostics and care to improve interventions and outcome in individuals with rare and complex epilepsies.

**EpimieRNA**
Aims to systematically investigate the occurrence and effects of microRNAs in humans and mice, and explores the potential of targeting microRNAs using designer drug-like molecules.

**EpiPGX**
Aims to identify genome-based biomarkers for use in clinical practice to individualise treatment of epilepsy, and stratify patients for clinical trials, aiming to avoid chemotherapy prevent relapse and reduce Adverse Drug Reactions (ADR). The project ended in 2015.

**EPISTOP**
Aims to better understand the pathophysiology of epilepsy and its consequences, to develop a preventative strategy for epilepsy, to identify new biomarkers of epilepsy, and to develop new therapeutic targets to block or otherwise modify epileptogenesis in humans.

**EPITARGET**
Focuses on the process leading to epilepsy (epileptogenesis) in adults. The project studies biomarkers and multiple basic mechanisms for epileptogenesis and translate these findings to the clinic by validating the biomarkers in human samples.

**Epixexchange**
Explored "Innovative Gene Therapies for Epilepsy Treatment", provided the bases for clinical application and implemented in the industrial arena new, advanced, unconventional strategies for the therapy of partial epilepsies. The project ended in 2015.
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