EUROCORES Programme
European Collaborative Research

Networking / Dissemination Activity
Scientific Report Form

REPORT FROM THE GENERAL ASSEMBLY OF EuroEINOMICS; Helsinki April 23-25, 2014

a) Summary

The General Assembly in Helsinki (venue for meeting and accommodation: Gustavelund Hotel http://www.gustavelund.fi/en) was the last joint assembly of the four Collaborative Research Projects (CRPs) of the EuroEPINOMICS consortium, funded for three years during 2011-2014. The aim of the meeting was to report the activities and obtained scientific results within individual CRPs to the whole consortium, to exchange ideas and, importantly, to discuss and plan future research projects and collaborations. Four scientific sessions highlighted the progress within each of the four CRPs, with the project leader giving a brief introductory overview in the beginning, followed by scientific talks partially chosen from the submitted abstracts and partially invited by project leaders. Outside these main sessions, specific time was dedicated to project specific internal discussions. A poster session, where mostly young investigators presented their work at the poster, was also organized with the posters being displayed through most of the meeting. Lunch and coffee breaks as well as joint dinners allowed time for discussion and interaction outside the scheduled sessions. The last session of the program was dedicated to a general discussion with emphasis on discussing opportunities for future research networks, collaborations and dissemination. A steering group meeting was organized mainly to discuss the use of the remaining project funds. Overall, the meeting was generally concluded to be successful, with emphasis on presentation of yet unpublished data, active cross-CRP interactions and discussions as well as plans for future.

b) Final programme of the event

Wednesday April 23, 2014 / Arrivals

19:00– Get together dinner at the restaurant in Gustavelund

Thursday, April 24, 2014

08:30–09:40 **SESSION 1: (Auditorium)**
Epiglia: Genetic Targets of Epileptogenesis and Pharmacoresistance in Brain Glial Cells
Chair: Erik Taubøll

08:30-08:40 Introduction and brief overview
(**Erik Taubøll,** Oslo)

08:40-09:00	Stimulation induced astrocytic Ca2+ signaling after status epilepticus in a mouse model of temporal lobe epilepsy (**Kjell Heuser**, Oslo)
09:00-09:20	Defining the role of impaired astrocyte K+ buffering in the generation of human temporal lobe epilepsy (**Peter Bedner**, Bonn)
09:20-09:40	Evidence for a role of Srp9 in mTLE and febrile seizure susceptibility (**Pierre de Graan**, Utrecht)
09:40–12:30	**SESSION 2 (Auditorium)** **CoGIE: Complex Genetics of Idiopathic Epilepsies** **Chairs: Holger Lerche and Bernd Neubauer**
09:40-10:00	Introduction and brief overview (**Holger Lerche**, Tübingen/IGE; **Bernd Neubauer,** Giessen/RE)
10:00-10:30	Analysis of IGE exome data - plans for further analysis with proper controls (**Holger Thiele**, Cologne; **Roland Krause**, Luxembourg)
10:30–11:00	Coffee break
11:00-11:25	CNVs from epilepsy exome data (**Kamel Jabbari**, Cologne)
11:25-11:50	*DEPDC5* mutations in idiopathic and focal epilepsies (**Yvonne Weber**, Tübingen; **Dennis Lal**, Cologne and Giessen)
11:50-12:15	Candidate gene analysis in IGE and Rolandic Epilepsy (**Yvonne Weber**, Tübingen; **Eva Reinthaler**, Vienna)
12:15-12:30	Synaptic and network changes in genetically absence epileptic rats from Strasbourg (**Zsofia Magloczky**, Budapest)
12:30–14:00	Buffet Lunch and Posters
14:00–15:20	**SESSION 3 (Auditorium)** **EpiGENet: Epigenetic Pathomechanisms Promoting Epileptogenesis in Focal and Generalized Epilepsies** **Chair: Asla Pitkänen**
14:00-14:10	Introduction and brief overview (**Asla Pitkänen**, Kuopio)
14:10-14:30	Genomic imprinting in genetic generalized epilepsies (**Ann-Kathrin Ruppert**, Cologne)
14:30-14:50	Comparison of DNA methylation alterations in three experimental models of epilepsy (**Katarzyna Lukasiuk**, Warsaw)
14:50-15:20	Circadian regulation of genes and proteins in control and epileptic mice (**Christophe Bernard**, Marseille and **Katarzyna Lukasiuk**, Warsaw)
15:20-16:00	Coffee break
16:00-18:30	**Internal discussion within projects** **(CoGIE+RES,** Auditorium**; EpiGENet,** big meeting room**; Epiglia,** small meeting room**)**
19:00–21:00	Buffet at the Krapi Restaurant

Friday, April 25, 2014 / Departures

08:00-08:30	**STEERING COMMITTEE MEETING**

08:30–09:45	**SESSION 3: EpiGENet continues (Auditorium)** **Chair: Asla Pitkänen**
08:30-09:00	Methylome characteristics and the non-coding genome (**Assam El-Osta**, Melbourne)
09:00-09:15	Metabolic interference with epigenetic alterations in a rodent model of TLE (**Katja Kobow**, Erlangen)
09:15-09:30	Profiling of methylome and transcriptome in experimental TBI in rats (**Noora Huusko**, Kuopio)
09:30-09:45	Molecular analysis of epileptogenic Ca2+-channel promoter regulation (**Karen Van Loo**, Bonn)
09:45-12:30	**SESSION 4 (Auditorium)** **RES: Genetics of Rare Epilepsy Syndromes** **Chair: Peter De Jonghe**
09:45-09:55	Introduction and brief overview (**Peter De Jonghe**, Antwerp)
09:55-10:10	E2 study (**Sarah Weckhuysen**, Antwerp)
10:10-10:25	Mutations in *XXXX* cause epileptic encephalopathy with similarities to Dravet syndrome and CSWS (**Johannes Lemke**, Bern)
10:25–11:00	Coffee break
11:00-11:15	*De novo* mutations in *HCN1* cause early infantile epileptic encephalopathy (**Stephanie Baulac**, Paris)
11:15-11:30	Clinical spectrum of epileptic encephalopathies associated with *SCN8A* mutations (**Rikke Steensbjerre Møller**, Dianalund)
11:30-11:40	Dravet and MAE: *de novo* dominant mutation analysis (**Arvid Suls**, Antwerp)
11:40-11:50	ESES: *de novo* dominant mutation analysis (**Bobby Koeleman**, Utrecht)
11:50-12:00	Dravet/NLES: recessive model analysis (**Tania Djémié**, Antwerp)
12:00-12:15	Recessive RES families: whole genome sequencing (**Katia Hardies**, Antwerp)
12:15-12:30	Saturation of the human genome with chromosomal breakpoints (**Niels Tommerup**, Copenhagen)
12:30-13:00	**Final Discussion and Concluding Remarks** **Chair: Holger Lerche**
13:00–14:00	Buffet Lunch
14:00–15:30	**SESSION 5: General discussion among PIs and APs (Auditorium)**

c) Description of the scientific content of the event (abstracts can be provided)

The first session presented achievements in the Epiglia project (Genetic Targets of Epileptogenesis and Pharmacoresistance in Brain Glial Cells), which is a translational research project aiming at unravelling the genetic and molecular pathways of temporal lobe epilepsy and febrile seizures. The leader of the CRP, Erik Taubøll's overview of the project was followed by three scientific presentations, representing three of the four subprojects. Kjell Heuser from Taubøll's group presented a project aiming at

understanding the role of astrocytic calcium signalling in a mouse model of temporal lobe epilepsy. Next Peter Bedner from Christian Steinhäuser's group presented their still ongoing work utilizing both animal models and human tissue samples and aiming at understanding the role of impaired potassium and glutamate buffering in the generation of temporal lobe epilepsy (see abstract Bedner et al.; abstracts as a supplement after list of participants). Finally Pierre de Graan, an associate partner from Utrecht, presented work in which genetic strategies in mice and humans combined with expression analysis in mouse models and in samples from human patients with febrile seizures have led to the identification of the Signal Recognition Peptide 9 gene as a putative candidate gene for febrile seizures (see abstract Hessel et al.).

In the second session progress in the CoGIE (Complex Genetics of Idiopathic Epilepsies), aiming at unravelling the genetic basis and pathophysiology of idiopathic generalized epilepsy and rolandic epilepsy, the two most common idiopathic epilepsy syndromes, was summarized. The two leaders of the main subprojects of the CRP, Holger Lerche (idiopathic generalized epilepsies) and Bernd Neubauer (rolandic epilepsy) first overviewed the progress and previous accomplishments in the exome and genome sequencing projects. The current status and future plans in both burden and candidate gene analysis of exome data, as well as analysis of genome sequencing data in families were presented in three talks, each given jointly by two persons, representing different groups, followed by a talk describing the efforts to analyse the exome data for copy number variations (see abstracts by Becker et al., Lal et al., Reinthaler et al. and Jabbari). The presentations evoked vigorous discussion, especially regarding a proper cohort of similarly exome sequenced control individuals and analysis strategies. Sequencing of such controls was near to be finished allowing in the near future proper data analysis and completion of the ongoing projects. The session was completed with a presentation by Zsofia Magloczky, who presented her group's data on expression analysis of epilepsy-associated proteins, with emphasis on synaptic proteins, in a rat model for epilepsy. These and other data by her group were presented in more detail in three posters of her young group members (see abstracts Nagy et al., Papp et al. and Toth et al.).

The third session highlighted work carried out in the EpiGENet (Epigenetic pathomechanisms promoting epileptogenesis in focal and generalized epilepsies) the general aim of which is to characterize common epigenetic pathomechanisms of epileptogenesis by utilizing both animal models and specimens from human brain. The CRP leader, Asla Pitkänen introduced and overviewed the project followed by seven scientific presentations the unifying theme of which was the application of state-of-art global analysis strategies to unravel epigenetic and/or gene expression alterations in human samples and rodent models of epilepsy. The first presentation focused on analysis of genomic imprinting in patients with idiopathic generalized epilepsies. The two following talks focused on rodent models and presented work aiming at identifying common changes and dissecting model specific differences in DNA methylation between three different experimental models of epilepsy and in circadian regulation of gene and protein expression. An associate partner, Assam El-Osta (see abstract) presented his work that has unravelled the role of non-coding RNAs in mediating remodelling of chromatin and specific histone modifications that impact gene expression patterns. The two following talks focused again on different experimental rodent models of epilepsy dissecting how metabolic interference affects epigenetic profiles and how traumatic brain injury affects DNA methylation and gene expression (see abstract Huusko et al.). Finally, the last talk in the session summarized work aiming at characterizing the regulation of expression of the T-type Ca^{2+} channel subunit $Ca_V3.2$, which has been identified as a

pivotal player in epileptogenesis in the pilocarpine model for temporal lobe epilepsy (see abstract van Loo et al.).

Finally, the fourth scientific session focused on the RES project (Genetics of Rare Epilepsy Syndromes), which aims to decipher the genetic basis of many rare epilepsy syndromes with emphasis on epileptic encephalopathies. The CRP leader Peter de Jonghe introduced the project in depth. He reviewed the past progress, including novel gene findings already published, as well as the current status and future plans, including description of networking beyond the EuroEPINOMICS consortium. This overview was followed by presentation of the first results of the E2 study, a collaborative effort between EuroEPINOMICS-RES and the Epi4K consortium in the U.S., which has resulted in identification on one further established novel gene and several strong candidate genes for epileptic encephalopathies (manuscript under review). In the two successive talks, two further novel gene findings were described, one of them still unpublished (Lemke, manuscript in preparation) and the other, HCN1, newly published (Baulac). Next, a clinical study on a cohort of patients with mutations in *SCN8A* was presented. The four following talks described the approaches taken in the analysis of exome and genome sequencing data in patients with various phenotypes under different inheritance models. Some potential novel candidate genes that had already emerged from theses studies were described and future plans were outlined. The presentations were followed by a lively discussion on future analysis strategies. The session was concluded with a talk given by an AP, Niels Tommerup (see abstract Tommerup), on a project aiming at using the previously identified chromosomal rearrangements to supplement the genome-wide sequencing strategies, which are the main approach taken in EuroEPINOMICS.

In addition to the scientific oral presentations, results were presented in seven posters (see remaining abstracts).

d) Assessment of the results and impact of the event on the EUROCORES programme.

The general assembly primarily served the purpose of informing the whole consortium on the obtained results and work in progress within the individual CRPs and subprojects, of creating new ideas, of further networking and, perhaps most importantly, of thinking ahead, beyond the termination of current funding. The scientific presentation with following discussions, both during the sessions and within the CRP-specific meeting served these purposes well.

A face-to-face meeting at this stage of the project was in particular very important for the CoGIE and RES CRPs as many projects are still in progress and will be completed after beyond the EuroEPINOMICS funding period. Future actions were discussed and decided upon.

An important forum for planning for future was the general discussion among all CRPS in the end of the meeting where the emphasis was on discussing the various funding options to enable continuation of work within the well-established and well-functioning network. During the duration of EuroEPINOMICS a huge amount of genomic data and tools have been produced, which will nurture future research provided that sufficient funding opportunities will be available. The different opportunities within Horizon 2020 were considered, discussed and decided to need further exploration and discussion.

e) List of speakers and participants

Anttonen	Anna-Kaisa	Folkhälsan Res Center, Helsinki, Finland
Barisic	Nina	University of Zagreb Med School, Crotia
Baulac	Stéphanie	ICM, Paris, France
Bedner	Peter	University of Bonn, Germany
Bernard	Christophe	Aix-Marseille Université, France
Caglayan	Hande	Bogazici University, Istanbul, Turkey
Craiu	Dana	Obregia Hospital, Bucharest, Romania
de Graan	Pierre	University Med Center Utrecht, The Netherlands
de Jonghe	Peter	VIB, University Antwerp, Belgium
Dębski	Konrad	The Nencki Institute of Experiment Biol, Poland
Djémié	Tania	VIB, Univ Antwerp, Belgium
El-Osta	Assam	Baker IDI, Australia
Guerrini	Renzo	Children's Hospital A. Meyer, Univ of Florence, Italy
Hardies	Katia	VIB, University Antwerp, Belgium
Heuser	Kjell	Oslo University Hospital, Norway
Hoffman-Zachrska	Dorota	Inst of Mother and Child, Warsaw, Poland
Huusko	Noora	University of Eastern Finland
Iliescu	Catrinel	Carol Davila University of Medicine, Bucharest, Romania
Jabbari	Kamel	University of Gologne, Germany
Joensuu	Tarja	Folkhälsan Res Center, Helsinki, Finland
Klein	Karl Martin	Epilepsy Center Hessen, Philipps-University Marburg, Germany
Kobow	Katja	Institute of Neuropathology, Erlangen, Germany
Koeleman	Bobby	NUMCU The Netherlands
Krause	Roland	University of Luxembourg
Laari	Anni	Folkhälsan Res Center, Helsinki, Finland
Lal	Dennis	University of Cologne, Germany
Lehesjoki	Anna-Elina	Folkhälsan Res Center, Helsinki, Finland
Lehesjoki	Annu	Folkhälsan Res Center, Helsinki, Finland
Lemke	Johannes	Institute of Human Genetics Leipzig, Germany
Lerche	Holger	Univ of Tübingen, Germany
Lindh	Sinikka	Folkhälsan Res Center, Helsinki, Finland
Linnankivi	Tarja	Folkhälsan Res Center, Helsinki, Finland
Lipponen	Anssi	University of Eastern Finland
Lukasiuk	Katarzyna	Nencki Institute of Experiment Biol, Warsaw, Poland
Maglóczky	Zsófia	Inst of Experiment Med, Hungarian Acad of Sci, Hungary
Maljevic	Snezana	Hertie Institute for Clinical Brain Research, Tübingen, Germany
Marini	Carla	Pediatric Hospital A. Meyer-University of Firenze, Italy
May	Patrick	LCSB, University of Luxembourg
Muona	Mikko	Folkhälsan Res Center, Helsinki, Finland
Nagy	Agoston	Inst of Experiment Med, Hungarian Acad of Sci, Hungary
Neubauer	Bernd	University of Giessen, Germany
Pal	Deb	King's College London, UK
Palotie	Aarno	FIMM, Helsinki, Finland
Papp	Peter	Inst of Experiment Med, Hungarian Acad of Sci, Hungary
Pernhorst	Katharina	Institute of Neuropathologie, Bonn, Germany
Pitkänen	Asla	University of Eastern Finland
Reinthaler	Eva	Medical University of Vienna, Austria
Ruppert	Ann-Kathrin	Cologne Center for Genomics, Germany
Sander	Thomas	Cologne Center for Genomics, Germany
Selmer	Kaja	Oslo University Hospital, Norway
Serratosa Fernandez	Jose M	Universidad Autónoma de Madrid, Spain
Sperk	Günther	Medical University Innsbruck, Austria
Steensbjerre Møller	Rikke	Danish Epilepsy Centre, Dianalund, DK

Štěrbová	Katalin	University Hospital Motol, Prague, Czech Rep.
Suls	Arvid	University of Antwerp, Belgium
Szocsics	Péter	Inst of Experiment Med, Hungarian Acad of Sci, Hungary
Talvik	Tiina	University of Tartu, Estonia
Tang	Shan	King's College London, UK
Taubøll	Erik	Oslo University Hospital, Norway
Terczynska	Iwona	Inst of Mother and Child, Warsaw, Poland
Thiele	Holger	Cologne Center for Genomics, Germany
Tommerup	Niels	University of Copenhagen, Denmark
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Van Loo	Karen	Institute of Neuropathology, Bonn, Germany
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Zimprich	Fritz	Medical University of Vienna, Austria
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APPENDIX: ABSTRACTS

Defining the role of impaired astrocyte K^+ and glutamate buffering in the generation of human temporal lobe epilepsy.

Peter Bedner, Julia Jordan, Gerald Seifert & Christian Steinhäuser

Institute of Cellular Neurosciences, University of Bonn, Bonn, Germany

In the sclerotic hippocampus of patients with mesial temporal lobe epilepsy (MTLE-HS), glial cells lose the glutamate-metabolising enzyme, glutamine synthetase (GS), and display reduced inwardly rectifying K (Kir) currents, which was speculated to contribute to generation and spread of seizure activity. It is, however, still an open question whether these alterations represent the cause or merely a consequence of the disorder. To specifically identify the effect of astrocytic Kir4.1 deletion on seizure generation in adult animals, we bred floxed Kir4.1 mice with Cx43kiCreERT mice, which express a tamoxifen-inducible Cre recombinase under control of Cx43 gene regulatory elements. Using single-cell real-time PCR and electrophysiological analyses, we found that after tamoxifen injection the majority of hippocampal astrocytes in the transgenic animals were devoid of Kir4.1 transcript and Ba^{2+}-sensitive K^+ currents. To investigate whether these mice are more prone to seizures, we subjected the mice to the unilateral intra*cortical* kainate injection model of MTLE-HS and observed by telemetric EEG recordings and videomonitoring the severity of the *status epilepticus*, the duration of the latent phase and the frequency of spontaneous seizures during the chronic phase. The results of this study are currently analyzed.

In a parallel approach, we investigated the impact of fibrile seizures (FS) on astroglial Kir channels function in human hippocampal slices. For this

purpose, astroglial Kir current densities from whole-cell patch-clamp experiments raised over a period of more than 10 years were re-analyszed and compared between MTLE-HS patients with and without antecedent FS. Intriguingly, the results revealed that patients with FS possess significantly lower astroglial Kir current densities, indicating an association between FS and Kir4.1 function.

In the second part of the project, we set out to evaluate seizure-induced changes of GS expression during the early phase of epileptogenesis. Using immunohistochemical staining and semiquantitative real-time PCR analysis we observed a pronounced reduction of GS protein and mRNA levels in hippocampal astrocytes aready three days after kainate injection in the mouse model of MTLE-HS. These findings point to a causative role of GS dysfunction in epileptogenesis.

Evidence for a role of Signal Recognition Peptide 9 (Srp9) in mTLE and febrile seizure susceptibility

Ellen V.S. Hessel[1], Henk Karst[1], Esther de Graaff[4], Hein A. van Lith[5], Ewart de Bruijn[6], Dick Lindhout[7,8], Carolien G.F. de Kovel[7], Bobby P.C. Koeleman[7], Marjan van Kempen[7], Eva Brilstra[7], Edwin Cuppen[6,7], Maarten Loos[9], Sabine S. Spijker[9], Peter C. van Rijen[2], Peter H. Gosselaar[2], Marian J.A. Groot Koerkamp[10], Frank C.P. Holstege[10], Cornelia van Duijn[11], Jeanette Vergeer[11], Henriette A. Moll [12], Erik Taubøll[13], Kjell Heuser[13], Geert M.J. Ramakers[1], Jeroen Pasterkamp[1], Onno van Nieuwenhuizen[3], Casper C. Hoogenraad[4], Martien J.H. Kas[1], and **Pierre N.E. de Graan[1]**

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The ethiology of febrile seizures (FS), the most common seizure type in young children, has a strong genetic component. Complex FS are a risk factor for mesial temporal lobe epilepsy (mTLE). By employing a phenotype driven genetic strategy using the C57BL/6J-Chr#A/NaJ chromosome substitution strain panel we mapped a quantitative trait locus (QTL) for hyperthermia-induced FS on mouse chromosome 1. Signal Recognition Particle 9 (*Srp9*) in the QTL was differentially expressed between parental strains, and its binding partner *Srp14* was co-identified as a strong candidate gene in a FS QTL on chromosome 2. The SRP complex plays a key role in the synthesis of membrane proteins, such as glutamate receptors. In vivo knock-down of brain Srp9 reduced FS susceptibility, thus

establishing causality between Srp9 levels and the phenotype. The mouse strain (CSS1) with reduced Srp9 expression and FS susceptibility, exhibited reduced hippocampal AMPA and NMDA currents. Down-regulation of Srp9 in hippocampal neurons reduced surface expression of AMPA receptor subunit GluA1. Consistent with a role of SRP9 in human FS, we detected increased hippocampal SRP9 expression in mTLE patients with antecedent FS. Comparing mTLE patients and healthy controls we found an association of a SRP9 promoter SNP (rs12403575 G/A) with FS and mTLE, which was confirmed in FS patients. Our findings identify SRP9 as a novel FS gene and implicate (local) ER-dependent protein synthesis and glutamate receptor expression in the mechanism. Pathways involving Srp9 may provide new leads for early diagnosis and treatment of children with complex FS and at risk for mTLE.

CNVs from epilepsy exome data

Kamel Jabbari

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Recently rare copy number variants (CNVs) have been recognized as important risk factors for both generalized and childhood epilepsies. We performed a systematic survey of CNVs derived from exome data of epilepsy patients to shed more light on the role in disease aetiology of this class of variants.

We analyzed 461 exomes from patients with Rolandic and adulthood epilepsies using an in-house pipeline that makes use of mapping and depth approaches to detect small and large (from 1kb up to 5Mb) CNVs. Validation was performed using multiple orthogonal methods: visual inspection of depth differences between cases and controls, array data when available and qPCR to validate small deletions.

We found that 68 patients out of 461, *i.e.* 14,7%, carried rare CNVs, several of which may contribute to epilepsy; others are known to be associated with the disorder. We also surveyed the literature for large deletions associated with epilepsy and could obtain a better resolution for some of them. We finally highlight additional novel candidate genes

DEPDC5 **mutations in idiopathic generalized epilepsy**

Felicitas Becker[1*],Stefan Wolking[1*], Julian Schubert[1*], Janine Altmüller[2], Holger Thiele[2], Pasquale Striano[3], Demet Kuscu[4],Hande Caglayan[5], Auli Sirèn[6], Thomas Sander[2], Wolfram Kunz[7], Anna-Elina Lehesjoki[8], Federico Zara[3], Peter Nürnberg[2], Holger Lerche[1], **Yvonne Weber**[1], EuroEpinomics Consortium

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DEPDC5 mutations are known to be causative in focal epilepsies like the familial focal epilepsy variable foci (FFEVF), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and familial temporal lobe epilepsy (FTLE), Rolandic epilepsy and related syndromes. In this study we investigated 249 patients from families with idiopathic generalized epilepsy syndromes for *DEPDC5* mutations and identified 7 novel *DEPDC5* variants in 15 families and two single cases with German, Turkish, Italian and Finnish origin. Four point mutations were predicted to be probably (p.E1337G, p.S1298P, p.P249S, p.R1405C), one possibly damaging (p.E891G) and two to be benign (p.A461T, p.V978G). One mutation, p.E1337G, occurred in 65% of all mutation-positive cases (11/17). Most of the families showed a homogeneous phenotype: 11 families had an absence epilepsy; 4 families had a juvenile myoclonic epilepsy. The mutations segregated well in all examined affected family members, although the co-segregation analysis has not been finished yet. Altogether, our findings suggest that mutations of *DEPDC5* contribute to idiopathic generalized epilepsy.

***DEPDC5* mutations in genetic epilepsies of childhood**

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Recent studies reported *DEPDC5* loss-of-function mutations in different focal epilepsy syndromes. Here we identified one predicted truncation and two missense mutations in three independent children with Rolandic epilepsy (3/207). In addition, we identified three families with unclassified focal childhood epilepsies carrying predicted truncating DEPDC5 mutations (3/82). The detected variants were all novel, inherited, and present in all tested affected (11) as well as in seven unaffected family members indicating low penetrance. Our findings extend the phenotypic spectrum associated with mutations in DEPDC5 and suggest that Rolandic epilepsy, albeit rarely, and other non-lesional childhood epilepsies are among the associated syndromes**.**

Analysis of *ELP4*, *SRPX2* and interacting genes in typical and atypical Rolandic epilepsy

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Purpose: Rolandic epilepsy is the most common idiopathic focal childhood epilepsy with a complex mode of inheritance. Two genes, *ELP4* and the X-chromosomal located *SRPX2* gene were postulated to confer susceptibility of RE and its atypical variants (ARE). To evaluate the contribution of these so far unreplicated genes to Rolandic Epilepsy we investigated a cohort of 280 patients.

Methods: We performed exome sequencing and SNP-array genotyping to screen for sequence and structural variants.

Results: We did not find an enrichment of rare disruptive mutations or copy number variations in *ELP4*, *SRPX2* or their functional interaction partners in our patients compared to European controls. We detected the known functional p.N327S variation in the *SRPX2* gene in two male patients and one female control. Including all control data the variant is seen with an overall similar frequency in cases and controls, albeit with a slight preponderance in male patients. We did not find the previously reported association of SNPs in the *ELP4* gene with centrotemporal spikes.

Conclusion: In conclusion, unlike past studies the data of this first reexamination analysis do not support a major role of *ELP4* and *SRPX2* in the etiology of Rolandic Epilepsy.

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Methylome characteristics and the non-coding genome

Assam El-Osta

Baker IDI

The findings we are presenting here strongly indicate a role for ncRNA dependent chromatin interactions in the regulation of gene transcription. While it has been known for years that epigenetic pathways control gene expression, the mechanism in the brain remains poorly defined. Examples are discussed showing ncRNAs mediate chromatin remodeling events and specific histone modifications that serve to regulate gene expression patterns. These findings will support the application of RNA-based approaches together with HDAC inhibition as a unique therapeutic strategy. With respect to ncRNA dependent chromatin interactions, I will explore long-standing questions relevant to our understanding of transcriptional control.

Post traumatic alterations on DNA methylome affects expression of epileptogenesis related genes

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Traumatic brain injury (TBI) is estimated to cause 10-20% of all acquired epilepsies. After the initial damage caused by direct mechanical force to the head, secondary damage develops over time consisting of molecular changes that underlie the subsequent reorganization of neuronal networks. The little evidence available suggests that epigenetic regulation controls some part of the alterations found in the expression of hundreds of genes after TBI. We hypothesize that DNA methylation alters gene expression, which regulates post-injury reorganization of neuronal circuits, eventually leading to the development of hyperexcitability and epilepsy. TBI was induced with lateral fluid-percussion injury to adult rats (n=5). Five sham-operated rats served as controls. At 3 months after TBI sampling of the hippocampus and cortex was done for DNA extraction. Sequencing of methylome was carried out with Illumina Genome Analyzer IIx. After TBI, methylation was changed in the gene body area in 21 genes in the hippocampus and in 45 genes in the cortex (adj.p-value<0.05). Three promising candidate genes with altered gene expression and methylation level were identified for further validation. Our results demonstrate long-lasting change after TBI in DNA methylation, which can explain altered gene expression levels of known epileptogenesis related genes.

Molecular analysis of epileptogenic Ca^{2+}-channel promoter regulation

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Temporal lobe epilepsy (TLE) is one of the most common seizure disorders in adults. The underlying mechanisms during its etiopathogenesis, collectively referred to as epileptogenesis, are however poorly understood. Recently, we have identified the pore-forming T-type Ca^{2+} channel subunit $Ca_V3.2$ as a pivotal player for epileptogenesis in the pilocarpine model of TLE. Here, we aimed to identify the molecular signaling cascades involved in *$Ca_V3.2$* transcriptional regulation. First, we determined the promoter region and observed several stimulatory and inhibitory clusters. Furthermore, we found binding sites for the transcription factor early growth response 1 (Egr1) and the zinc sensor metal-regulatory transcription factor-1 (MTF1) to be highly overrepresented within the *$Ca_V3.2$* promoter region. mRNA expression analyses and dual-luciferase promoter assays revealed that the *$Ca_V3.2$* promoter was strongly activated by the two transcription factors. Congruently, whole-cell I_{CaT}s were significantly larger after Egr1 and MTF1 overexpression. Furthermore, rAAV-mediated overexpression in mice hippocampi *in vivo* caused $Ca_V3.2$ upregulation, whereas overexpression of a dominant-negative variant of Egr1 or MTF1 significantly reduced the pilocarpine-induced $Ca_V3.2$ upregulation. Thus, Egr1 and MTF1 can regulate *$Ca_V3.2$* promoter activity and mRNA expression and hence, the size of I_{CaT}. These findings may provide new possibilities for pharmacological intervention aimed at epigenetic and/or promoter regulation for preventing the process of epileptogenesis.

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Saturation of the human genome with chromosomal breakpoints

Niels Tommerup, on behalf of the The Breakpoint Mapping Consortium

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Even in the era of exome and full genome sequencing, it will take decades and tremendous resources to saturate the human genome with mutations linked to abnormal and normal phenotypes.

As a supplement to exome and full genome sequencing strategies, we will use already identified balanced chromosomal rearrangements (BCR) to establish a first, detailed map of mutations covering a significant fraction of the human genome. In the first reexamination of *unselected de novo* BCRs (BCRdn) detected by 40 years of prenatal diagnosis in Denmark, we have shown that BCRs truncate protein coding genes, ncRNA genes, unannotated transcripts detected by deep sequencing, as well as developmental regulatory genomic landscapes, mimicing random mutagenesis. In addition, our study revealed a ~20% long-term disease-risk of unselected BCRdn, 2-3 fold higher than previously assumed, and exclusively associated with early- and later-onset neurodevelopmental disorders. The unselected cohort also revealed a fundamental lack of functional knowledge for probably a majority of our genes, and that a genotype-phenotype relationship can be obtained for a significant number of the human genes by mapping of BCRs.

We have initiated clinical reexamination/mapping of all known BCRs in Denmark. Based on a population of just 5.5 mill, this will provide data on ~1.000 breakpoints alone. Presently, >60 participants from 30 countries and 6 continents have joined the consortium, supporting the vision to reach a proposed first goal of ~10.000 breakpoints. Unlike other large scale genomic efforts, all countries including undeveloped and developing countries can participate. The breakpoint map will identify and confirm numerous genotype-phenotype associations, saturate the regulatory landscapes around evo-devo genes that specify the vertebrate body plan, reveal novel genetic mechanisms, and define genomic regions, which can be mutated without any direct phenotypic consequence.

Comparison of DNA methylation events in three animal models of temporal lobe epilepsy

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DNA methylation is recently postulated to play a crucial role in gene regulation involved in epileptogenesis. We explore next generation sequencing data obtained from three animal models of temporal lobe epilepsy (TLE) (amygdala stimulation induced - aSE, pilocarpine induced SE - pilo, Traumatic Brain Injury - TBI) at 3 mo after induction of epilepsy to find common changes in DNA methylation occuring in epileptic hippocampus at chronic state. We assigned differentially methylated regions ($p < 0.01$) to multiple genomic features (CpG Islands, SNPs, most conserved sequences, promoters, TSS, 5'UTRs, Exons, Introns, Gene Bodies, 3'UTRs, and 5kb downstream regions).

Distributions of differentially methylated regions differs among the genomic features and it seems to be a model specific. When models were compared in pairs there was 20 methylated regions common between aSE and pilo, 24 common regions between aSE and TBI and 29 between pilo and TBI.

Similarities in transcriptome alternations between hippocampus and cortex during post traumatic epileptogenesis

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Traumatic brain injury (TBI) is a significant cause of acquired epilepsies. After the initial damage by direct mechanical force to the head, secondary brain damage leads to molecular changes affecting reorganization of neuronal networks in epileptogenesis. We hypothesize that expression of genes that control formation of neuronal circuits alters network features after TBI in both, hippocampus and cortex, leading to increased seizure susceptibility and epilepsy.TBI was induced with lateral fluid-percussion injury to adult rats (n=5). Five sham-operated rats served as controls. At 3 months post-TBI two 2-mm-thick coronal slices were sectioned to sample the hippocampus and cortex for RNA extraction. Sequencing of transcriptome was carried out with Illumina Genome Analyzer IIx. In the hippocampus, 4280 genes and cortex 176 genes were expressing differently when compared to controls (adj.p<0.05). The expression of 140 same genes was altered significantly in both brain areas. Most common functional terms in among of these differential expressing genes were glycoprotein, signal, plasma membrane and ion/metal/cation binding. Our result reveals long-lasting alternations after TBI in gene expression in the hippocampus and cortex.

Investigation of expression levels and distributions of different synaptic proteins in hippocampi, cortices and thalami of Genetic Absence Epilepsy in Rats from Strasbourg

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In our experiment with Genetic Absence Epilepsy Rats form Straßbourg (GAERS) we were aiming to show the presence of distinct synaptic proteins which play an essential role in the synaptic membrane fusion process between transmitter carrying vesicles and the presyanptic membrane. The gene of one of the examined proteins was found to be in mutant form in idiopathic epileptic patients, therefore we decided to investigate it's expression in various brain regions, like hippocampus, cortex and thalamus. The rats were perfused under deep anesthesia, afterwards 60 um sections were cut, and immunohistochemistry was performed. With light microscopic analysis we have found presumable mossy fiber sprouting in the CA3 subfield of the Ammon's horn, however, there was no sign of sprouting in the granular layer of the dentate gyrus. The presence of this phenomenon of temporal lobe epilepsy in genetic epilepsy suggest overlapping mechanisms in different epileptic disorders. For exact localization of synaptic proteins in different brain areas immunogold reaction was prepared. Light- and electron microscopic analyses of the distribution of the proteins are in progress.

Investigation of expression levels and distributions of calcium-binding proteins and SP receptor expression in Genetic Absence Epilepsy in Rats from Strasbourg (GAERS)

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Several lines of evidence suggest the existence of interplay between the neuropathological mechanisms of temporal lobe epilepsy (TLE) and absence epilepsy. In the current study, we aimed at investigating the expression levels and distribution of the calcium-binding proteins calretinin (CR), parvalbumin (PV) and calbinin (CB), as well as the substance P receptor (SPR) in the thalami, hippocampi and cortical areas of Genetic Absence Epilepsy Rats from Strasbourg (GAERS) using immunocytochemistry. Light microscopic analyses of GAERS samples revealed a significant reduction in the number of CR- and PV-immunopositive interneurons in the hilar hippocampal subfield, as well as in the S1 and barrel cortices. Calbindin expression showed an inhomogeneous pattern with mildly faded immunostaining of hippocampal granule cells and stratum moleculare in some epileptic animals, but nearly control-like staining in others. No significant alteration was found in the expression levels of SPR at the light microscopic level as compared to non-epileptic Wistar controls. Analyses of the same marker proteins in thalamic nuclei and the electron microscopic validation of our preliminary findings are in progress.

TLR4, ATF-3 and IL8 inflammation mediator expression correlates with seizure frequency in human epileptic brain tissue

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Inflammatory processes in the central nervous system (CNS) may have modulatory effects on seizure frequency as previously shown in animal models. In human patients only little is known about differential expression of inflammatory factors in correlation to seizure frequency and other clinical parameters. Brain tissue from pharmacoresistant patients with mesial temporal lobe epilepsy (mTLE)

provides a unique prerequisite for clinico-neuropathological correlations. We have concentrated on gene expression and epigenetics of the human key inflammatory mediators, TLR4, ATF-3 and IL8, in correlation to seizure frequency in human epileptic brain tissue of pharmacoresistant mTLE patients.

We isolated total RNA and DNA from human biopsy brain tissue of pharmacoresistant mTLE patients (n = 26). We performed whole-genome expression profiling using Illumina Human HT-12 v3 Expression BeadChips. We further used Illumina HumanMethylation450 BeadChips for the detection of quantitative methylation state of 450K CpG sites. Corresponding human hippocampal sections for immunohistochemistry were stained with antibodies against TLR4, ATF-3, IL8 and glial fibrillary acidic protein (GFAP), NeuN and the microglial marker HLA-DR.

We observed that abundant *TLR4* expression correlated significantly with high seizure frequency. For *ATF-3,* we found an inverse correlation of expression to seizure frequency. Lower expression of *IL8* was significantly associated with high seizure frequency. Furthermore, epigenetic analyses point to inverse correlation between methylation of distinct promoter motifs and TLR4 expression. TLR4 expression was mainly in neurons and GFAP-positive astrocytes. Only neurons express ATF-3, microglia and astrocytes IL8.

Our results suggest a differential correlation of inflammatory key factor expression in epileptic hippocampi and seizure frequency in patients.

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Changes in the Expression of Histone Deacetylase 1-11 mRNAs in the Hippocampus in Two Mouse Models of Temporal Lobe Epilepsy

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Gene expression can be regulated epigenetically by DNA methylation and post-translational histone modification. Histone modifications involve deacetylation of histone proteins contributing to transcriptional silencing of gene expression.
Deacetylation of histone proteins is carried out by HDAC families of enzymes, which are classified in four different classes comprising 11 HDAC isoforms. We investigated changes in the expression of HDAC mRNAs in two animal models of temporal lobe epilepsy.

We adapted a mouse model for TLE using local injection of kainic acid (KA; 0.350 nmol/70 nl; deep ketalar/isofluorane anesthesia) into the hippocampus. EEGs were continuously recorded subduraly for 4 weeks. After an initial status epilepticus (lasting for 3 days), the animals exhibited about 2 spontaneous seizures per day. In the injected hippocampus, losses in CA1 and CA3 pyramidal cells are observed after 24 hrs and granule cell

dispersion after 14 days. The mice were killed at different intervals (4, 12, 24, 48 hrs, and 14 and 28 days) after KA injection and their brains were removed and frozen. Coronar sections were obtained in a microtome and subjected to in situ hybridization for HDAC 1-11 mRNAs.

In the dentate gyrus, expression of HDAC 1, 2, 7 and 11 mRNAs was significantly decreased 4 hrs after KA ipsi- and contralaterally to the injection, recovering after 48 hrs. In contrast, HDAC5 mRNA levels were significantly increased 4 and 12 hrs after KA injection, remaining increased in the injected hippocampus at later intervals. There was also a pronounced increase in HDAC9 mRNA expression 14 and 28 days after KA restricted to the injected dentate gyrus. For HDAC3 we observed a transient increase in mRNA levels after 24 to 48 hrs (contralateral) and a decrease of HDAC4 mRNA levels after 24 hrs (ipsi-and contralateral).

Our data show distinctly different and specific expression patterns for the different HDAC mRNAs indicating rather specific changes in the expression of numerous genes after KA-induced seizures. The early bilateral decreases in HDAC 1, 2, 7 and 11 mRNAs may be caused by the initial status epilepticus and may be related to the rapid expression of various genes as described earlier. Subsequently increased expression of HDACs 2 and 3 may counteract this reaction. Overexpression of HDAC9 mRNA may be associated with the granule cell dispersion developing concomitantly. To investigate this further we also established a mouse model using i.p. injection of pilocarpine. The mice also develop a status epilepticus with subsequent spontaneous seizures, but no granule cell dispersion. In situ hybridization in these mice revealed similar decreases in HDAC 1 to 3 but no increases in HDAC 5 and 9 at the late intervals. This observation supports the idea that the decreases in HDAC 1 to 3 are related to the initial status epilepticus and that the changes in HDAC 5 and 9 at the injection site relate seen in the KA model to granule cell dispersion. Supported by the Austrian Research Fund Project I 664).

Investigation of Density Changes of Citrate Synthase in Hippocampi of Patients with Temporal Lobe Epilepsy

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Dysfunction of mitochondria is well known in several neurological disorders and in different types of epilepsy. There is a limited amount of evidence about changes of the density of mitochondrial enzymes in human epileptic samples, and the available data are controversial. Therefore, we examined

surgically removed hippocampal samples from 18 patients with drug resistant temporal lobe epilepsy (TLE). Post mortem control samples were obtained from 5 subjects without any sign of neurological disorders. Immunohistochemistry was used to assess the density of citrate synthase, which is a widely accepted method for detecting intact and functional mithocondria. Light and electron microscopic analyses were carried out. In control tissue differences in the homogeneity of principal cellular staining was found among hippocampal subfields. In contrast, epileptic tissue exhibited inhomogeneous staining patterns whithin specific subregions, like CA3 and dentate gyrus. Elevated intensity of citrate synthase-immunostaining was observed in the CA2 area of each epileptic hippocampus. In previous studies this region was found to be a spontaneous spike generator in TLE, providing a possible explanation for the presence of the high rate of mitochondrial metabolism. Quantitative analyses with Image J program was carried out.