Complex genetics of idiopathic epilepsies (CoGIE)

Goal:
To unravel the genetic background and pathophysiology of common idiopathic epilepsy syndromes:
- Idiopathic generalized epilepsy (IGE)
- Rolandic epilepsy with centrotemporal sharp waves (RE)

Tools:
- clinically well characterized families with IGE and RE
- whole exome/genome next generation sequencing
- copy number variation and GWAS
- bioinformatic analysis
- Functional validation
**Section B – Individual Projects (IPs)**

**IP1 (Peter Nürnberg, Germany)**  
*Title of Project*: Whole-exome sequence screening for rare variants predisposing to idiopathic generalized epilepsy.

**IP2 (Anna-Elina Lehesjoki, Finland)**  
*Title of Project*: High-throughput genotyping of candidate sequence variants for IGE identified by next generation sequencing.

**IP3 (Bernd Neubauer, Germany)**  
*Title of Project*: Genomic variation in Rolandic epilepsy.

**IP4 (Fritz Zimprich, Austria)**  
*Title of Project*: Multiple rare variants in Rolandic epilepsy.

**IP5 (Rudi Balling, Luxembourg):**  
*Title of Project*: Bioinformatic and computational analysis of sequence variants associated with common idiopathic epilepsies.

**IP6 (Holger Lerche, Germany):**  
*Title of Project*: Functional studies with automated patch clamping of selected variants in ion channel or transporter genes

**IP7 (Zsófia Maglóczky, Hungary):**  
*Title of Project*: Neuronal expression pattern of proteins affected in IGE and RE.
Section C – Associated Projects (APs)

AP1 (Aarno Palotie, UK):
*Title of Project:* Whole exome sequencing in 95 IGE and 50 RE families.

AP2 (Bobby PC Koeleman, The Netherlands):
*Title of Project:* Targeted next generation sequencing of 250 candidate genes in 95 IGE families.

AP3 (Federico Zara, Italy):
*Title of Project:* Genome-wide and candidate gene-specific high resolution analysis of copy number variation (CNV).

AP4 (Thomas Sander, Germany):
*Title of Project:* Genome-wide association study for idiopathic generalized epilepsy.

AP5 (Steven Petrou, Australia):
*Title of Project:* Automated functional analysis of genetic variation in epilepsy.

AP6 (Dimitri Kullman, UK):
*Title of Project:* Functional validation of selected genetic variants using in vivo and in vitro virally mediated gene expression.

AP7 (Massimo Mantegazza, France):
*Title of Project:* Functional analysis in transfected cell lines and neurons.
Complex genetics of idiopathic epilepsies (CoGIE)

NGS: next generation sequencing
HT-SNP-GT: high throughput genotyping
CNV: copy number variation
GWAS: genome-wide association studies
Whole genome sequencing of whole IGE families (WGS):
WGS: pilot project with 10-20 IGE families: ≥ 4 clear IGE cases* (maximum 1 case with EGTCA accepted, others CAE/JAE or JME, mixed phenotypes possible)
WGS-2 (later, if possible): 3 homogeneous clear IGE cases* (only CAE/JAE or JME)
(1 index case goes for WES now)
WGS-3: 3 heterogeneous clear IGE cases* (1 case with EGTCA accepted)

Whole exome sequencing of >300 IGE index cases (WES):
WES: IGE families with ≥ 2 affected first degree relatives* with CAE**/JAE or JME (i.e. two different groups with absence or myoclonic seizures; in case of heterogeneous families, as 1 CAE + 1 JME: sequence both)
WES-2: 1 CAE/JAE or JME* + 1 IGE or only GSW on EEG or more distant second affected family member
WES-3: only EGTCA*
(Epicure phenotyping sheets, modified including PGX data)

*all counted as clearly affected IGE cases should have GSW on EEG
**EOAE accepted if no additional symptoms like movement disorder/mental decline
E1, Lehesjoki Helsinki

FAMILY E1_Lehesjoki Helsinki

J105

17MYO/18GTCS>JME/EKG:gen spw/PPR4

J106

J107

12MYO/27GTCS/JME/EKG:gen spw/PPR+grade?

J108

13MYO/13GTCS/JME/EKG:gen spw/PPR+ grade?

J109

AS/17GTCS/JAE/EKG:gen spw/PPR+ grade?

J110

Sent for WGS 08/2011
CAE56 (NRXN1+del)

WES
WGS-2
IGE 82

WES

WGS-3

CAE 4y, EGTCS 62y
L528

CAE 4y, EGTCS 31y
L507

EGTCS in childhood, EEG pos.

EGTCS in childhood, EEG pos.

L540

L541

L542
IGE228 mixed IGE

WES-2

L2436

L2463

L2458

EGTCS 14
JME 14y
L2457

L2461

FS 1x, 2y
L2460

CAE 4y
EGTCS 18y
L2459

1xFS in childhood
1 provoked EGTCS

L2449

L2448

L2462

L2450

1xFS
Further recruitment efforts!
Complex genetics of idiopathic epilepsies (CoGIE)

Sequencing centers:
- Sanger Institute
- Cologne Center for Genomics (CCG)
- Cooperation: Luxembourg Center for Systems Biology (LCSB), Complete Genomics (CG), Institute for Systems Biology (ISB), Seattle: for whole genome sequencing

Bioinformatic analysis:
- Sanger and CCG: primary data analysis (SNP calling, validation)
- CG/ISB with LCSB: data analysis of WGS in families
- Luxembourg Center for Systems Biology (LCSB): secondary data analysis (network effects, protein prediction)
Computational molecular and systems approaches to identify disease-related SNPs

Luxembourg Centre for Systems Biomedicine
Multilayer approach to study diseases

Sequencing Techniques
(GWAS, Next Generation Sequencing, Copy number Variations)

Mapping on cellular networks

Statistical Analysis

disease-related variants

Molecular analysis

disease-related variants

frequency of variants in health and disease

<table>
<thead>
<tr>
<th>Rank</th>
<th>SNP Loci</th>
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<tr>
<td>1</td>
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<td>2</td>
<td>chr1:248000001</td>
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Predicting the effect of mutations in proteins
Distribution of the Effects of Missense cSNPs on Protein Molecular Function for the SNP-Disease

- Stability 83%
- Binding 5%
- Catalysis 0%
- Allosteric response 1%
- Posttranslational modification 1%
- No effect 10%
Higher level maps of GWAS genetic interactions

- ADH1, PRC1
- PRP19 splicing factors
- 60S ribosomal biogenesis
- Small nuclear ribonucleoprotein complex
- 20S proteasome
- Mitochondrial RNA splicing
- BOI2, CDC24
- Pre-replication complex
- Mannan Polymerase II
- Tim9-Tim10 complex
- TRAPP complex
- Synaptosomal complex
- Actin-associated motorproteins
- Trehalose-6-phosphate synthase/ phosphatase complex
- Kornberg’s mediator (SRB) complex
- Actin-associated proteins
- Membrane actin-binding
- Oxidant-induced cell-cycle arrest
- 66S pre-ribosomal particles
- Bud neck kinase
- Vps27/Hse1 complex
- Cbf1/Met4/Met28 complex
- holo TFIIH complex
- DNA polymerase alpha (I) - primase complex
- Cdc48p-Npl4p-Ufd1p AAA ATPase complex
- FUN19, KAP123, YPR174C

Functional Enrichment of Traits:
- Green: Mitochondrial
- Red: Ribosomal
- Orange: Amino-acid metabolism
- Blue: Lipid metabolism
- Yellow: Oxidative stress
- Cyan: Sugar metabolism
- Purple: Translation
- Black: No enrichment
Epicure Project

Subprojects 1/WP3 and 2/WP3:
1. Collection of 95 families with IGE:
   - CAE, JAE, JME, EGMA/EGTC, all with typical seizures and GSW/PSp/PSW on EEG
   - ≥2 affected individuals with IGE
     + ≥1 with GSW on EEG

2. Sanger sequencing of 70 ion channel genes

3. Functional validation with electrophysiological techniques
Epicure Project

Subprojects 1/WP3 and 2/WP3 results so far:

1. >50 novel variants in 70 ion channel genes, not detected in
   - 800 European controls
   - dbSNP
   - available 1000 genome data

2. Frequency of such novel/private variants in the normal population?

3. Only partial cosegregation for many variants
   > expected for complex inheritance

4. Functional significance
   - Polyphen2 prediction ranging from benign to probably damaging
   - functional validation reveals several alterations in
     channel function that could explain a neuronal hyperexcitability
   - further analysis pending
Automated platforms for functional analysis

Roboocyte

Patchliner
16 distinct subunits classified into 7 subunit classes: \( \alpha(1-6), \beta(1-3), \gamma(1-3), \delta, \epsilon, \rho, \theta \)

most abundant subunit composition in brain
\( 2\alpha_1, 2\beta_2, 1\gamma_2 \)
Immunohistochemistry to localize proteins, example of sodium channels:

1. $\text{Na}_v1.1$ ($SCN1A$) is the Na channel in inhibitory neurons (knock-in SMEI mouse model)

Ogiwara et al. J Neurosci 2007
2. Age-dependent seizures due to transient expression of the sodium channel Na\textsubscript{v}1.2 during postnatal development.

Liao et al., Brain 2010
Genomic variation in typical and atypical Rolandic epilepsy

> We performed candidate gene analysis in about 80 sibpairs with Rolandic epilepsy, atypical Rolandic epilepsy and the associated EEG trait.

> In a small number of cases sequence variations in KCNQ2, KCNQ3, ACHRNA7, and SCN1A were detected. One family showed coding variants in KCNQ3 and SCN1A. A conclusive understanding and extension of these findings will only be possible by use of new comprehensive molecular genetic tools, like whole exome sequencing and CNV analysis.

> This is now planned in 113 small multiplex families and about 35 trios.
Genomic variation in typical and atypical Rolandic epilepsy in KCNQ2 and KCNQ3

(KCNQ2_c.523-525del, Lys116del) (KCNQ2_rs1801545)

(KCNQ2_c.1953>G, Ile592Met) (KCNQ3_c.1120G>A, Glu299Lys)

(KCNQ3_c.1367C>T, Ala381Val) (KCNQ3_c.1945C>T, Pro574Ser)

(KCNQ3_c.2309C>T, Pro695Leu) (KCNQ3_c.2488G>A, Asp755Asn)

(Neubauer et al. Neurology 2008)