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).

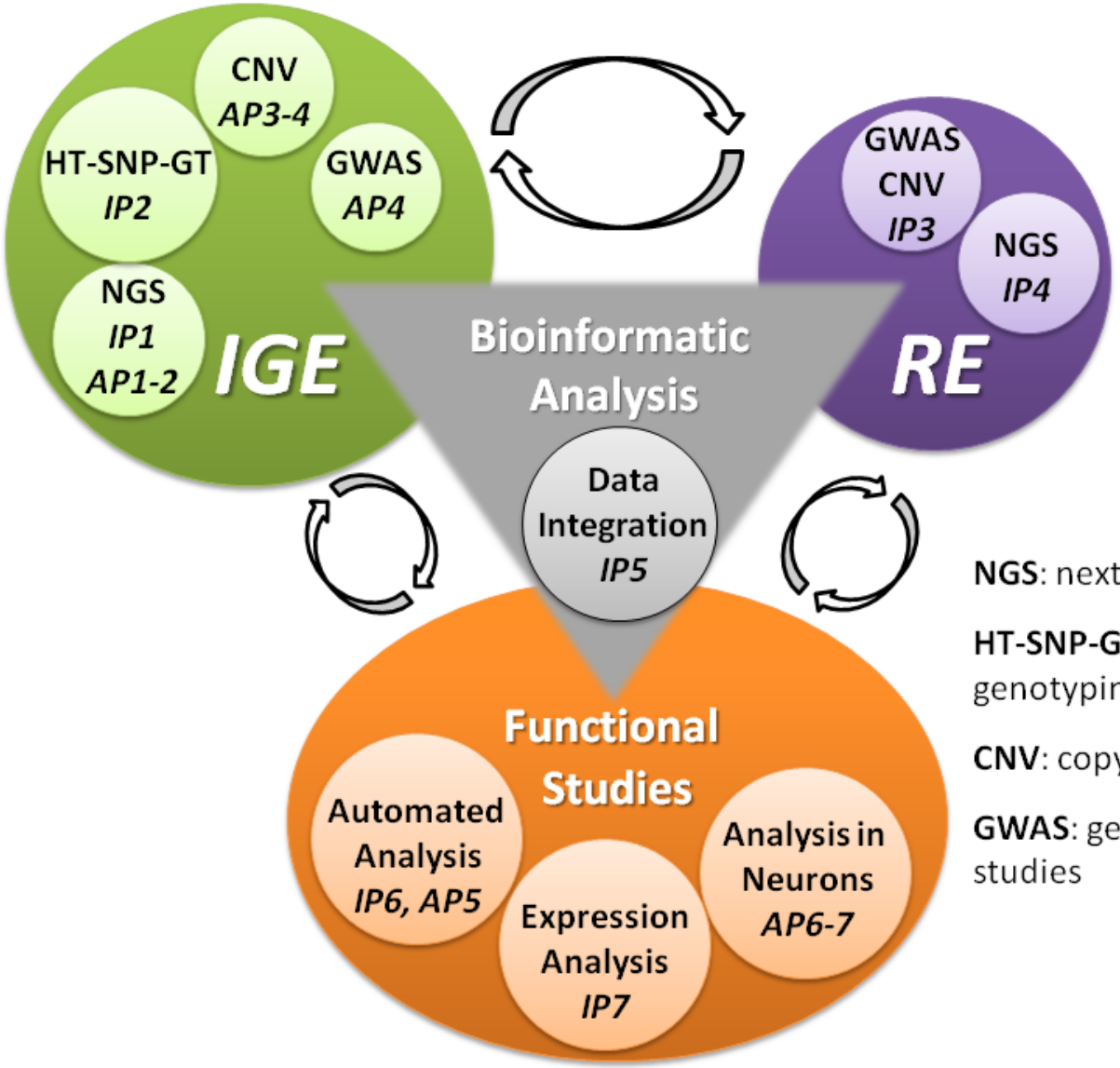
AP4 (Thomas Sander, Germany):
Title of Project: Genome-wide association study for idiopathic generalized epilepsy.

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

IGE phenotyping board San Servolo/Rome 2011

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

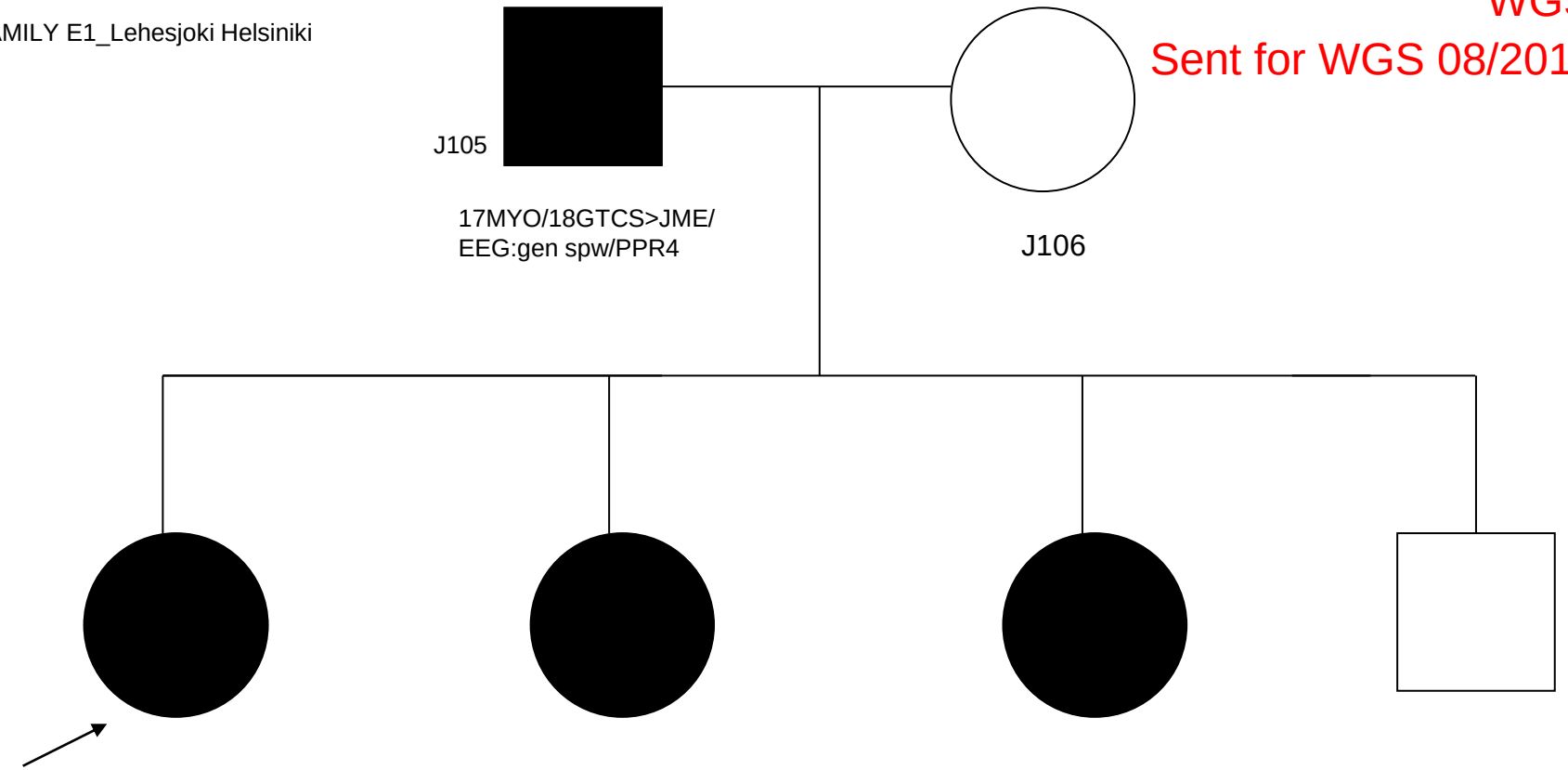
**EOAE accepted if no additional symptoms like movement disorder/mental decline

E1, Lehesjoki Helsinki

FAMILY E1_Lehesjoki Helsiniki

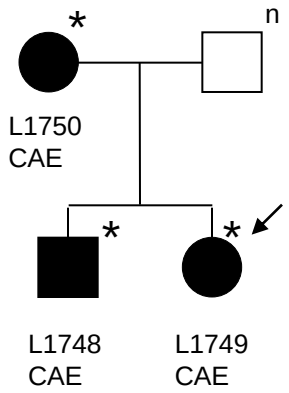
WGS

Sent for WGS 08/2011



CAE56 (NRXN1+del)

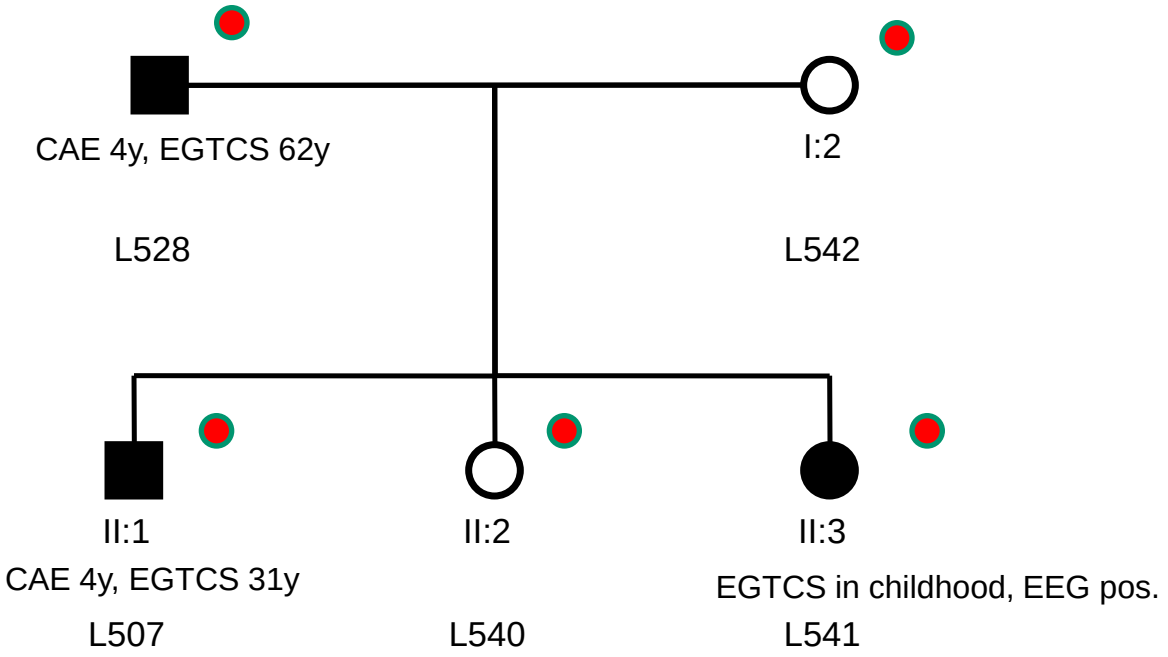
WES
WGS-2



IGE 82

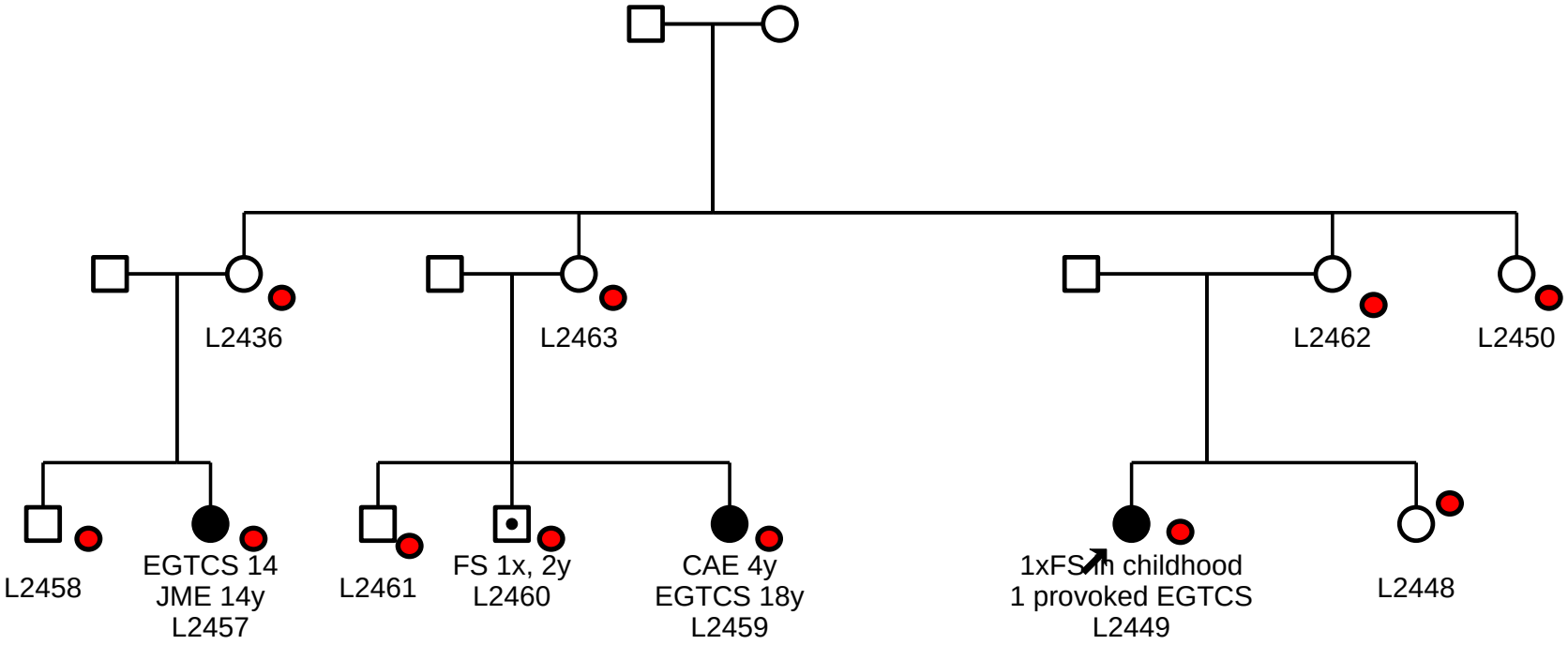
WES

WGS-3



IGE228 mixed IGE

WES-2



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:

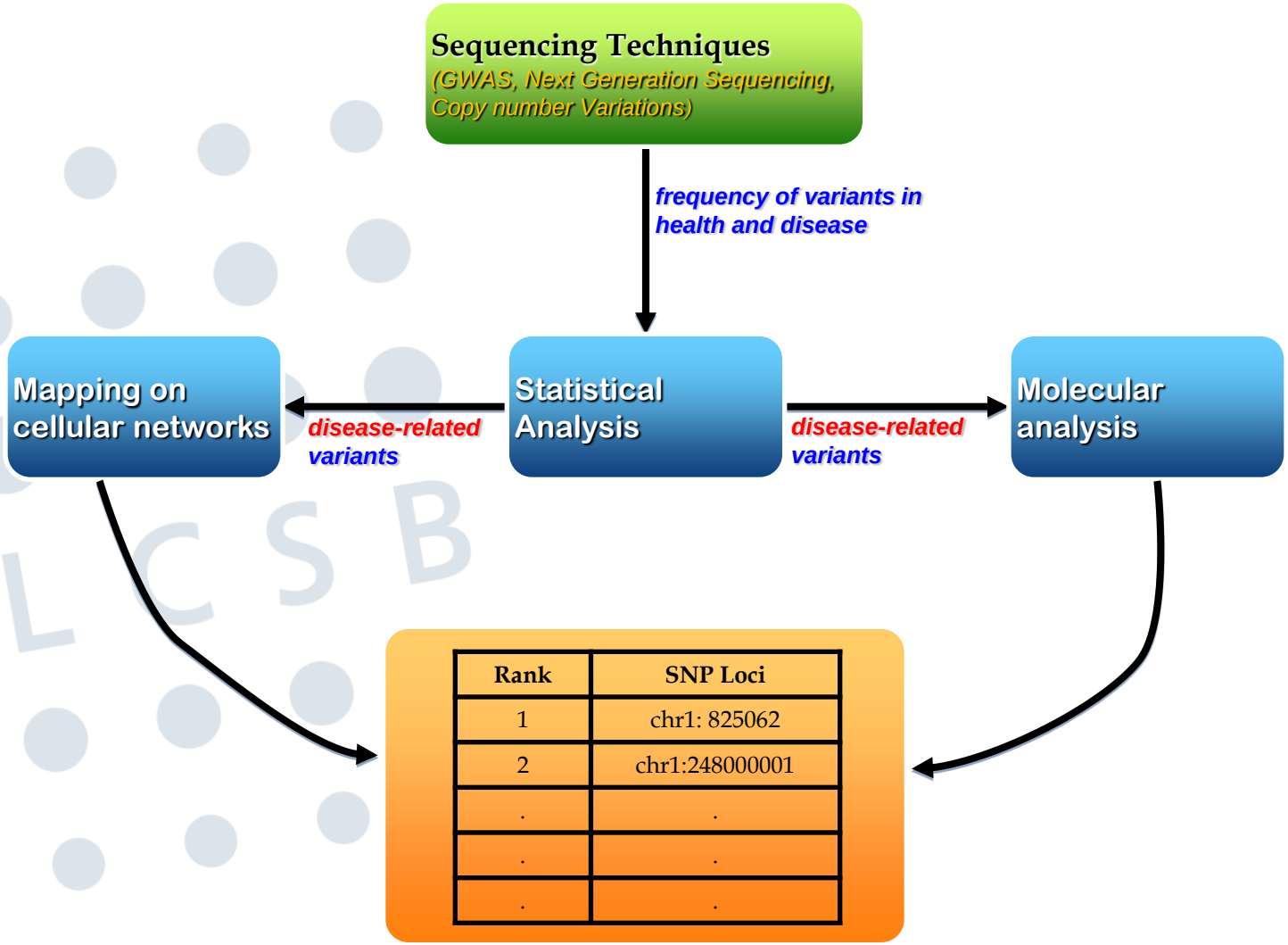
- Sanger and CCG: primary data analysis (SNP calling, validation)
- CG/ISB with LCSB: data analysis of WGS in families
- Luxembourg Center for Systems Biology (LCSB): secondary data analysis (network effects, protein prediction)

Computational molecular and systems approaches to identify disease-related SNPs

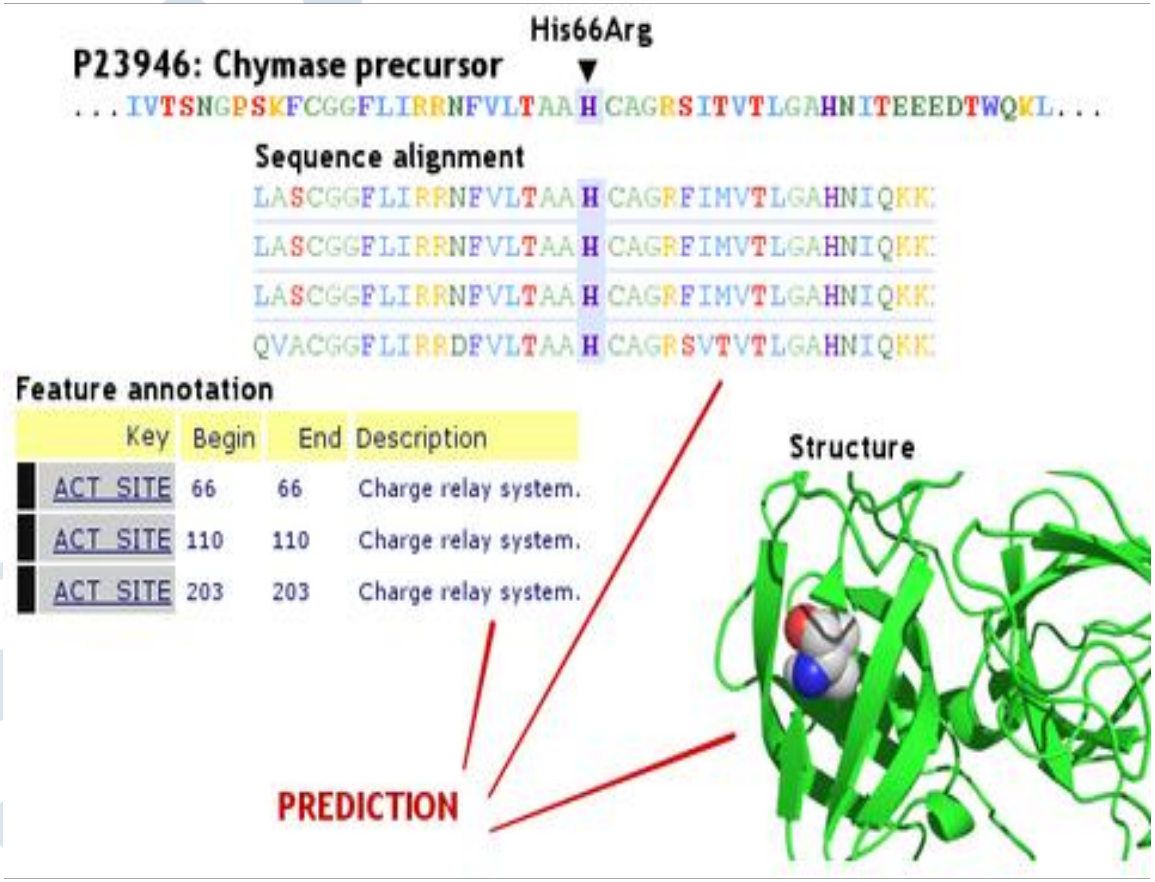
Luxembourg Centre for Systems Biomedicine

September 2011

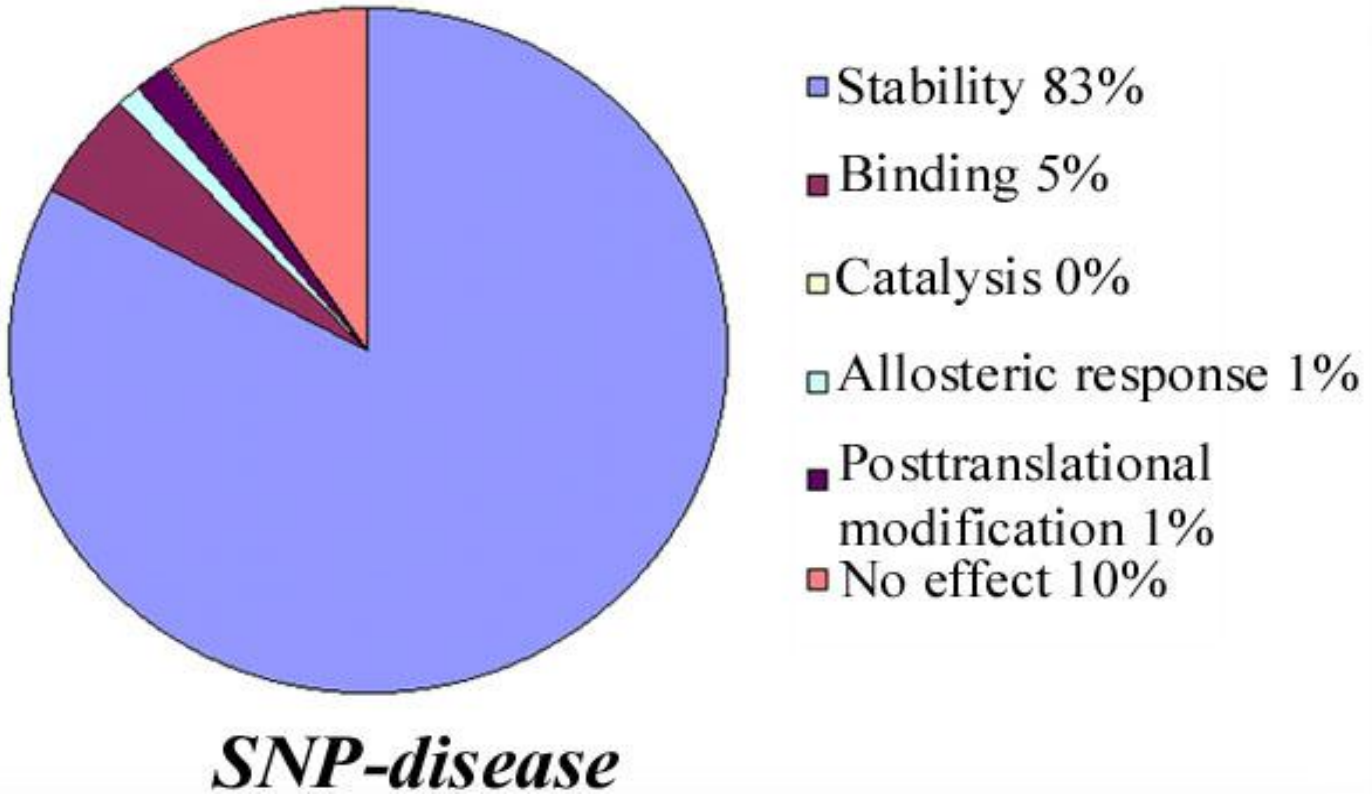
Multilayer approach to study diseases



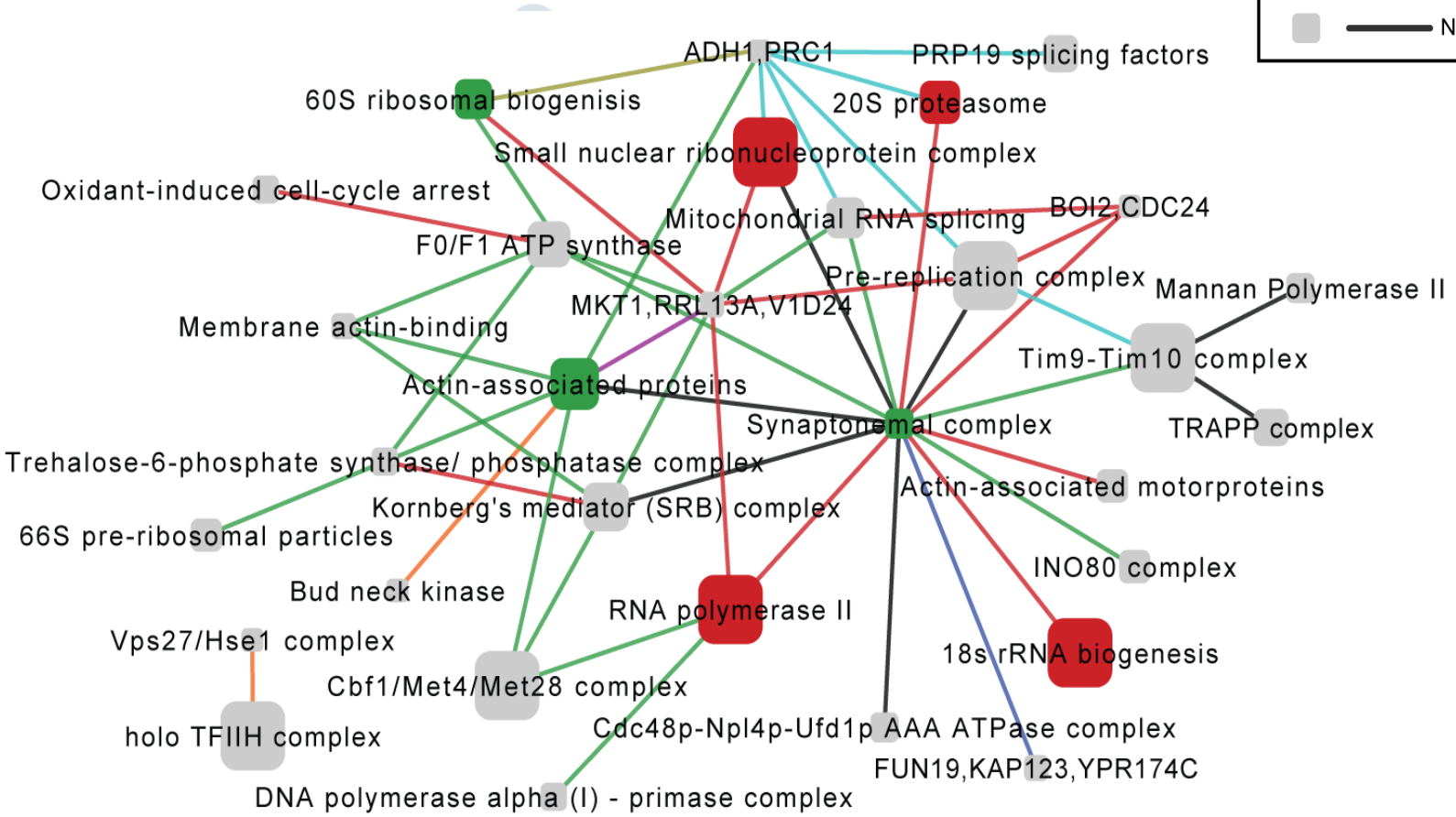
Predicting the effect of mutations in proteins



Distribution of the Effects of Missense cSNPs on Protein Molecular Function for the *SNP-Disease*



Higher level maps of GWAS genetic interactions



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:

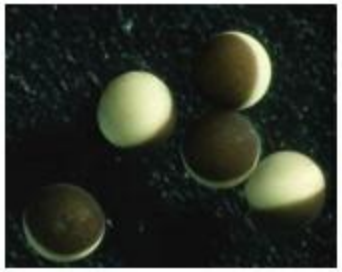
1. >50 novel variants in 70 ion channel genes, not detected in
   - 800 European controls
   - dbSNP
   - available 1000 genome data

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

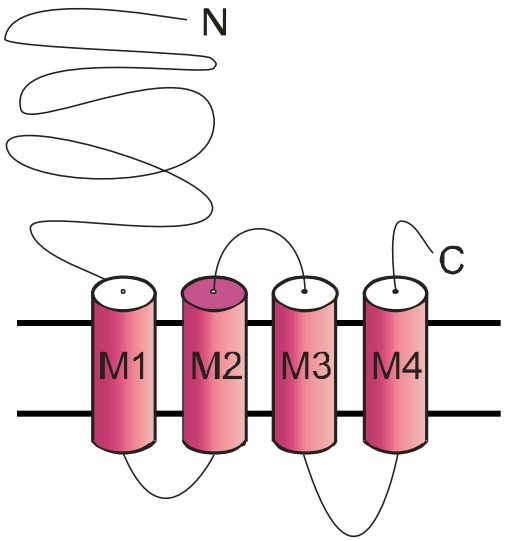
Automated platforms for functional analysis

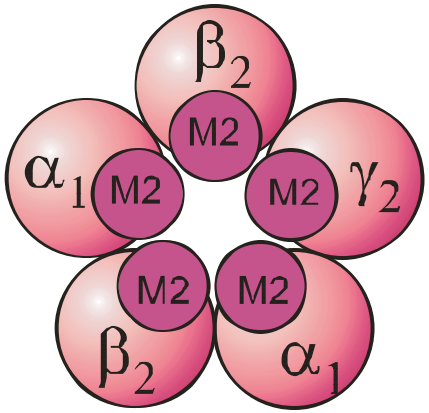


Roboocyte

Patchliner

GABA(A) Receptors



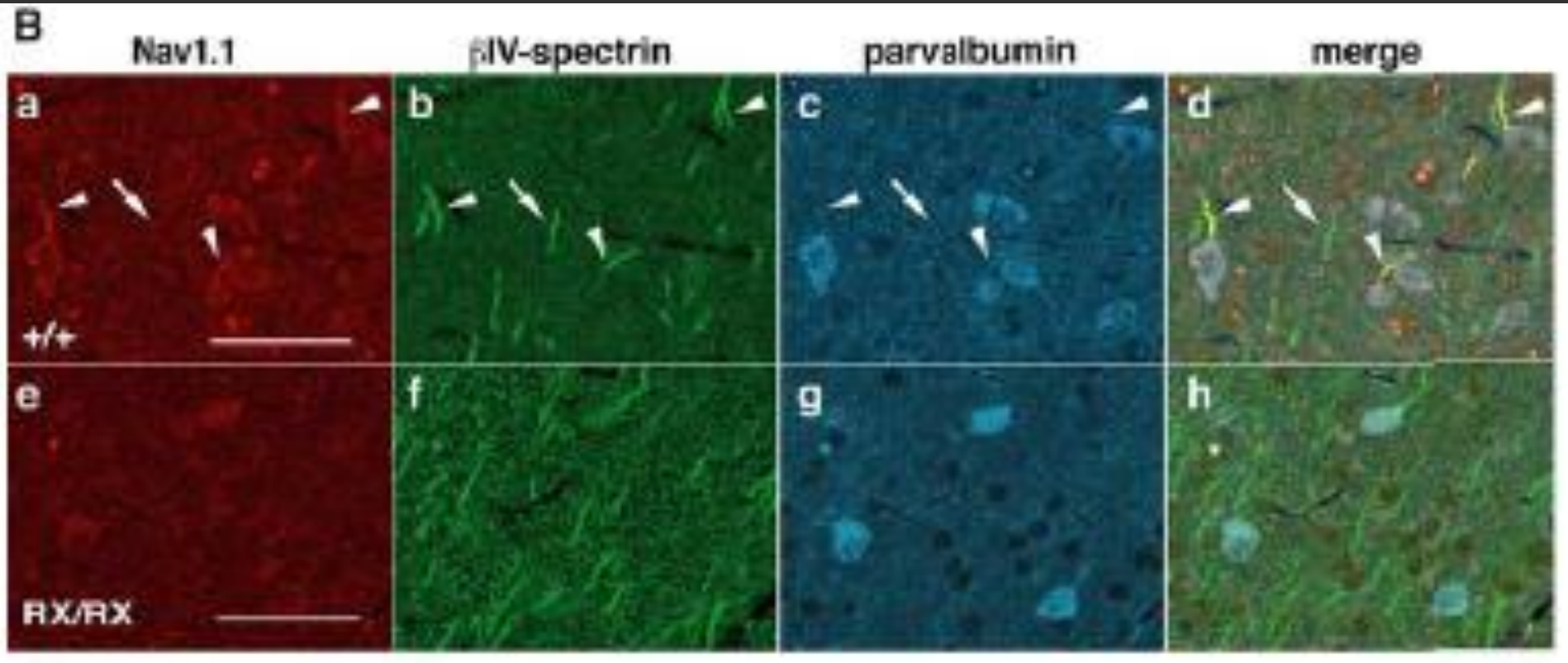


- 16 distinct subunits classified into 7 subunit classes:
  α(1-6), β(1-3), γ(1-3), δ, ε, ρ, θ

- most abundant subunit composition in brain
  $2\alpha_1, 2\beta_2, 1\gamma_2$

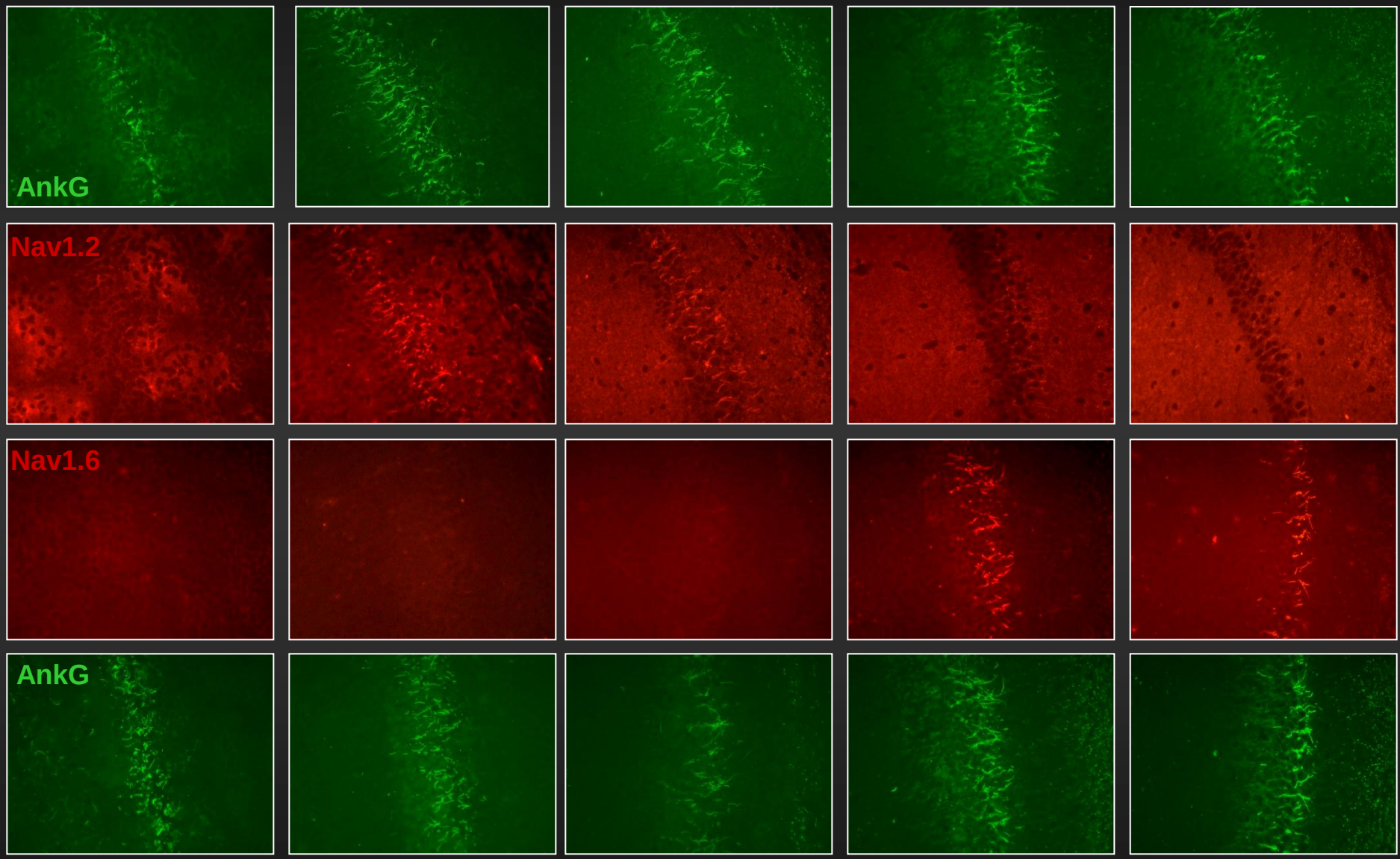
Immunohistochemistry to localize proteins, example of sodium channels:

1. $Na_v1.1$ (*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 $Na_v1.2$ during postnatal development



Liao et al., Brain 2010

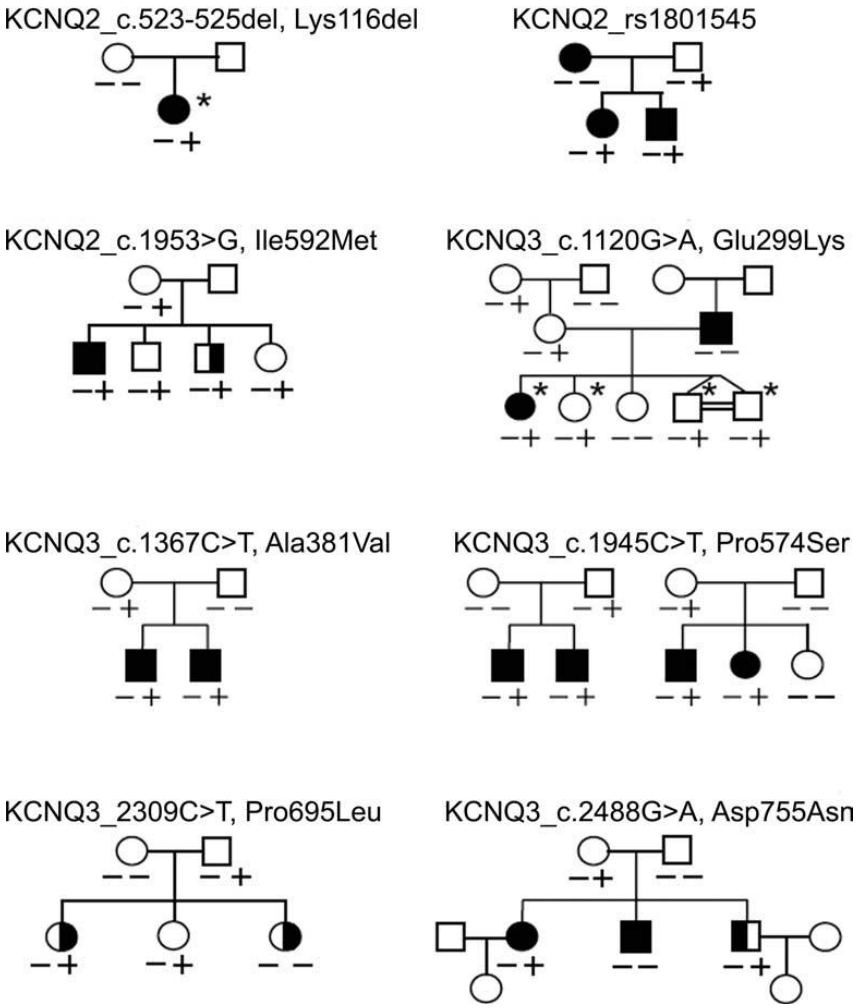
Genomic variation in typical and atypical Rolandic epilepsy

> GWe 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



(Neubauer et al. Neurology 2008)