European Science Foundation

Eurocores Project; EuroEpinomics

Kick-off meeting

September 20th 2011; Strasbourg

Genetics of rare epilepsy syndromes

Are these "rare" epilepsy syndromes really rare?

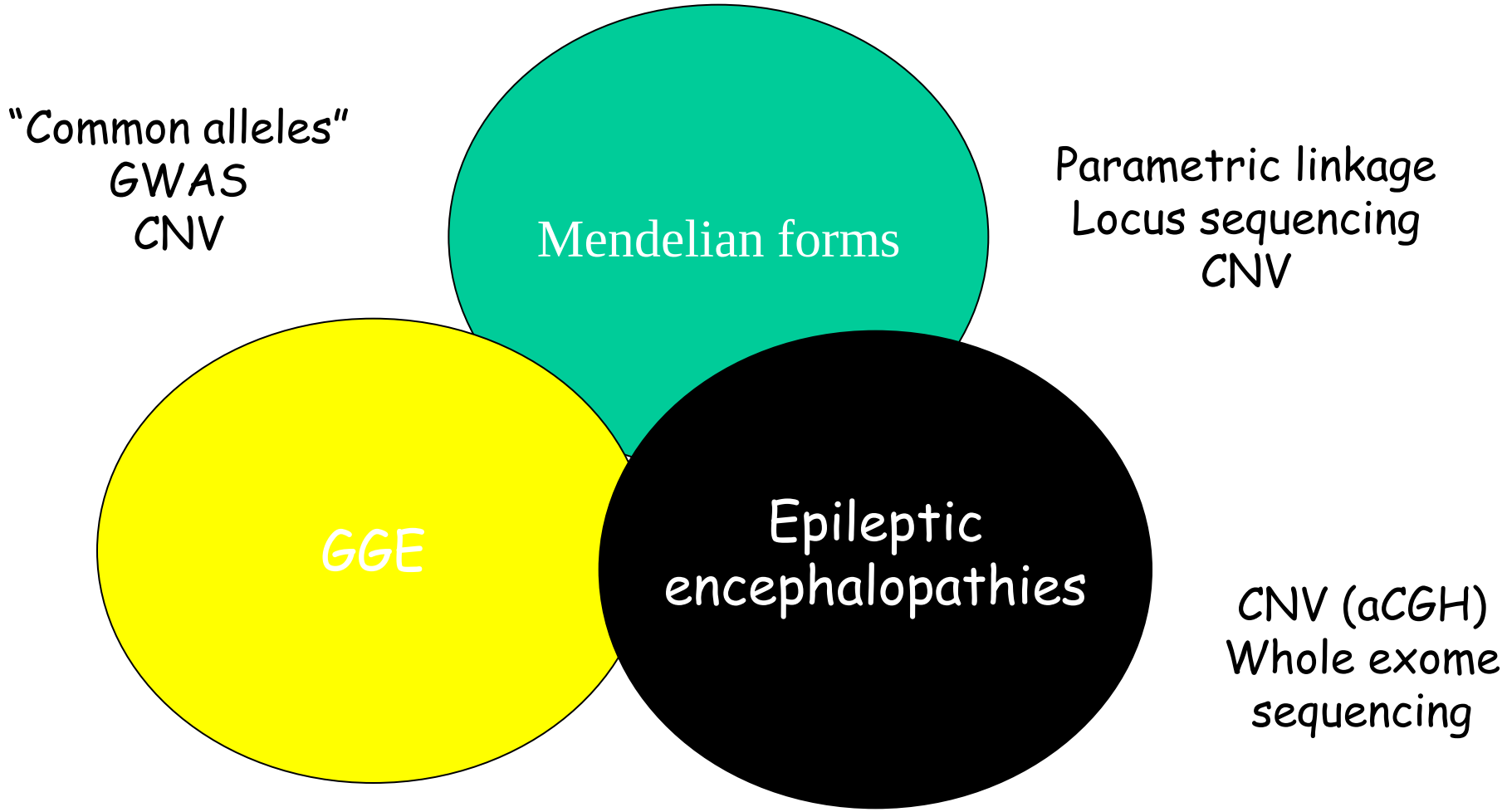
- Individually "rare" but "as a group "common"

Where do these monogenic "rare epilepsy syndromes" hide?

- Families
  - Autosomal dominant
  - Autosomal recessive
  - X-linked

- Isolated, early onset epilepsies

MONOGENIC

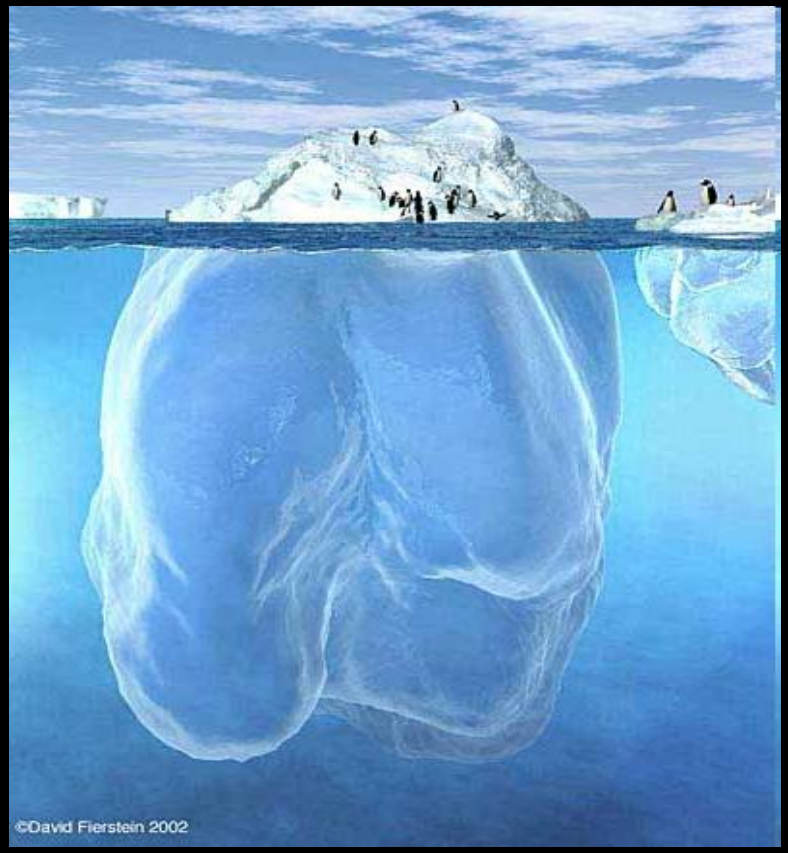

"Common alleles"
GWAS
CNV
Mendelian forms
Parametric linkage
Locus sequencing
CNV
GGE
Epileptic
encephalopathies
CNV (aCGH)
Whole exome
sequencing

SCN1A channelopathies

Generalized epilepsy with febrile seizures plus (GEFS+)

Missense mutations: altered kinetics

Families



Loss of function

De novo
Missense mutations
Nonsense mutations

Dravet Syndrome; SMEI

Isolated patients

whole gene deletions = CNV

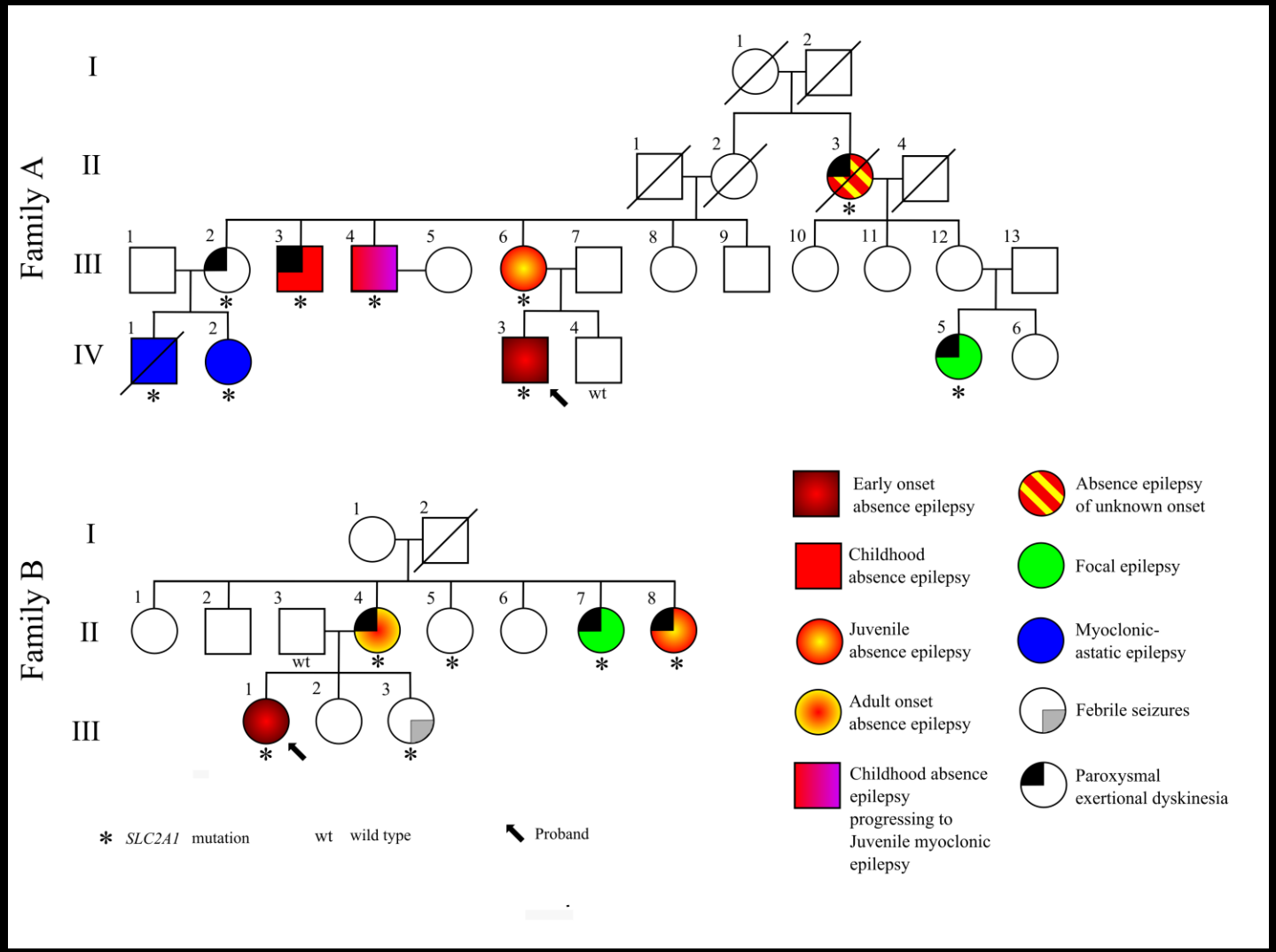
Early onset absences epilepsy

Table. Clinical and Mutation Details of Early-Onset Absence Epilepsy Patients with GLUT1 Mutations

Patient No.	Sex	Current Age	Age of Onset			Examination			Outcome		Family History of Epilepsy	*SLC2A1* Mutations			
			Absence	GTCS	Myoclonus	Motor	Head Circumference	Paroxysmal Dyskinesia Onset	Intellect	Seizure		Location in Gene	DNA Mutation	Protein Mutation	Position in Protein
1	F	28 yr	3 yr	7 yr	—	Normal	Normal	—	Normal	Seizure free with VPA from 7 years	Yes	Exon 5	c.668G>C	p.R223P	Loop TMD6 and TMD7
2	M	28 yr	3 yr	8 yr	—	Mild upper limb ataxia	Normal	—	Mild ID (IQ, 56)	Daily absences on VPA + TPM	Yes	Exon 7	c.971C>T	p.S324L	TMD8
3	F	12 yr	14 mo	—	14 mo	Mild gait ataxia	Normal	Subtle, 5 yr	Moderate ID (IQ, 48)	Sporadic absences on VPA + LTG	No Exon 4	c.376C>T	p.R126C TMD4		
4	F	7 yr	13 mo	12 mo	—	Normal	Normal	—	Normal	(IQ78)	Frequent absences on VPA+ LTG+ETX	No Intron 5	c.680-11G>A	p.227-228ins PPV	Loop TMD6 and TMD7

DNA mutations were numbered using the reference sequence NM_006516.1.
GTCS = generalized tonic-clonic seizure; VPA = valproic acid; TMD = transmembrane domain; ID = intellectual disability; IQ = intelligence quotient; TPM = topiramate; LTG = lamotrigine; ETX = ethosuximide.

SLC2A1 mutations in IGE



Aims

- 1. Achieve a molecular genetic diagnosis in rare epilepsy cases
  - Better and easier diagnosis
  - Prognosis
  - Genetic counselling
  - Enable pharmacological studies in homogeneous patient groups…

Broader Aims … links with other CRPS

- 2. Novel genes and pathways
  - Novel insights in seizures and epileptogenesis
  - Construction of cellular and animal models
  - Identification of novel therapeutic targets
    - Seizures
    - More disease specific

Partners

- Caglayan Hande; Istanbul; Turkey
- Craiu Dana; Bucharest; Romania
- Helbig Ingo; Kiel; Germany
- Hoffman-Zacharska Dorota; Warsaw; Poland
- Lemke Johannes; Bern; Switzerland
- Rosenow Felix; Marburg; Germany
- Selmer Kaja; Oslo; Norway
- Serratosa José; Madrid; Spain
- Talvik Tiina; Tartu; Estonia
- De Jonghe Peter; Antwerpen; Belgium

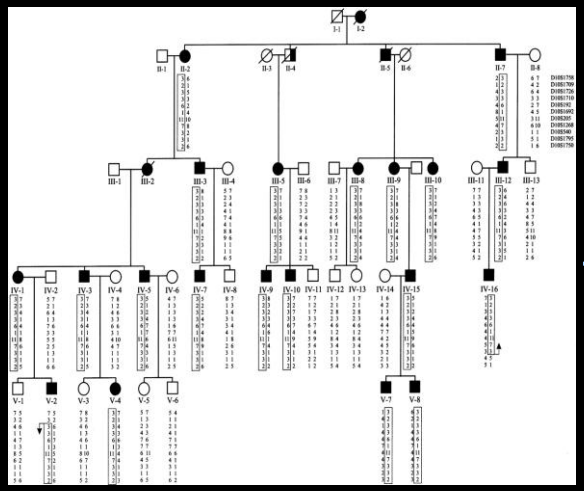
Associated Partners

- Lerche Holger; Tübingen; Germany
- Mefford Heather; Seattle; USA
- Tommerup Niels; Copenhagen; Denmark,
- Hjalgrim; Copenhagen, Denmark
- Barisic Nina; Zagreb; Croatia

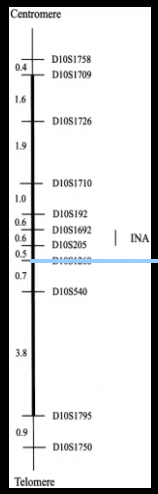
Expertise

- Patient populations
  - Large Index families/ trios
  - Follow up cohorts
- In depth phenotyping
  - Clinical; electrophysiology; imaging (MR)
- Technology platforms
  - Large capacities
  - High troughput
  - Complementary technologies

Positional Cloning

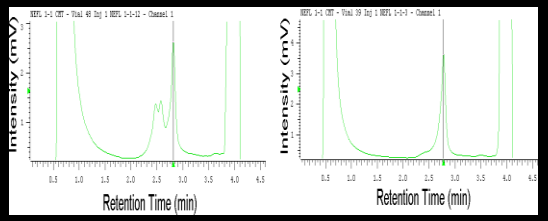


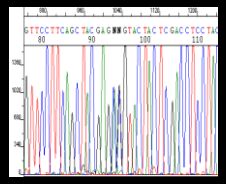
Genome search

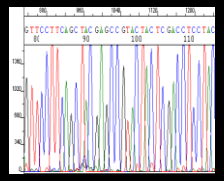


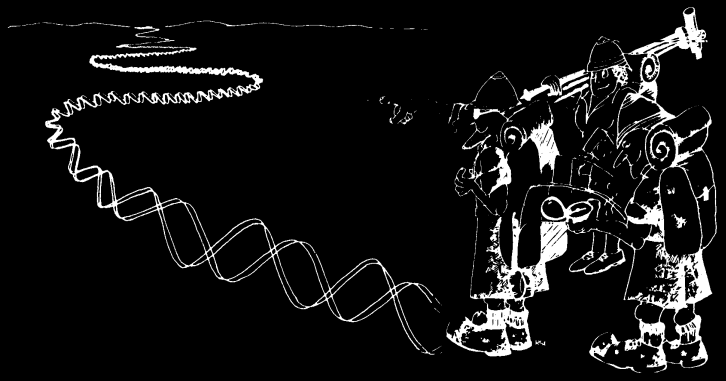
chromosome

Gene → mutation









Designation	*Locus*	*Chromosome*	*Gene*
Benign familial neonatal seizures (BFNS)	EBN1	20q13.3	*KCNQ2*
	EBN2	8q24	*KCNQ3*
	EBN3	5p15-q11	-
	unknown ≈ 30%	-	-
Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)	EFNL1	20q13	*CHRNA4*
	EFNL2	15q24	-
	EFNL3	1q21	*CHRNB2*
	EFNL4	8p21	*CHRNA2*
		3p22-p24	-
		8q11.2-q21.1	*CRH*
	unknown > 80%	-	-
Lateral temporal lobe epilepsy (ADLTE)		10q23–26	*LGI1*
	unknown ≈ 50%	-	-

Chromosome	*Phenotype/Syndrome*	*No of families*	*Model of inheritance**	*LINKAGE Program*	*Max Lod Score*	*Reference*	*Replication studies*
19q	BFIS	5	ad	-	> 8	Guipponi et al. 1997	
16p12–q12	BFIS	7	AD	MLINK	3.32	Caraballo et al. 2001	3
2q24	BFIS	8	AD	ALLEGRO	6.29	Malacarne et al. 2001	
1p36.12-p35.1	BFIS	1	AD	not determined	3.27	Li et al. 2008	
16p12–q12	ICCA	4	AD	MLINK	6.76	Szepetowski et al 1997	2
16p11.2-q12.1	PKC	8	AD	VITESSE	11.51	Tomita et al. 1999	3
16p12–q11.2	Rolandic epilepsy +PED+writers'cramp	1	AR	MLINK	3.68	Guerrini et al.1999	
2p24	GEFS+	1	AD	MLINK	4.22	Audenaert et al. 2005	
21q22	GEFS+	1	AD	FASTLINK	3.35	Hedera 2006	
5q14.3-q23.1	GEFS+	1	AD	FASTLINK	3.12	Deprez 2006	
3p23-p24	GEFS+	1	AD	LINKMAP	4.82	Nabbout et al. 2007	
18p11.2	GEFS+	1	AD	LINKMAP	2.02	Nabbout et al. 2007	1
6q16.3-q22.31	GEFS+	1	AD	SIMWALK2	4.68	Poduri et al. 2008	
8p23-p21	GEFS+	5	AD	MLINK	3.00	Baulac et al. 2008	
17q12-q24	GEFS+	1	AD	ANALYZE	2.84	Siren et al. 2010	
8q24	IGE	10	model free	-	3.40	Zara et al. 1995	
2q36	IGE	130	model free	GENEHUNTER	2.98	Sander et al. 2000	
14q23	IGE	130	model free	GENEHUNTER	3.28	Sander et al. 2000	
18q21	IGE	91	AR	GENEHUNTER	4.50	Durner et al. 2001	
5p15	absence seizures	91	AR	GENEHUNTER	3.80	Durner et al. 2001	
8p11	IGE, non JME	91	AR	GENEHUNTER	3.80	Durner et al. 2001	
9q32–33	IGE (GTCS)	1	AR	ALLEGRO	2.9	Baykan et al. 2004	
10q25–q26	GTCS in JME	11	model free	GENEHUNTER	4.20	Puranam et al. 2005	
10p11	IGE	20	AD	GENEHUNTER	4.23	Kinirons et al. 2008	
8q24	CAE	1	AD	MLINK	3.60	Fong et al. 1998	1
15q14	JME	34	AR	GENEHUNTER	4.42	Elmslie et al. 1997	
6p21	JME, IGE	24	AR	LIPED	3.04	Greenberg et al. 1988	5
5q12–q14	JME	1	AD	GENEHUNTER	3.33	Kapoor et al. 2007	
2q23.3, 2q24.1	JME	1	AR	MERLIN	2.62	Layouni et al. 2010	
2q33–q36	JME	1	AD	GENEHUNTER	9.92	Ratnapriya et al. 2010	
8q23-24	BAFME	1	AD	MLINK	5.42	Mikami et al 1999	1
2p11.1–q12.2	BAFME	2	AD	-	3.32	de Falco et al 2003	2

* AD = autosomal dominat, AR= autosomal resessive

Questions raised

- Are we really so succesful?
- Why is the pace of gene-discovery slowing down?

- Potential hurdles:
  - Some loci may be false positive
  - Mistakes in delineation of linkage intervals
  - Loci to big to handle with classical Sanger sequencing
  - Unusual mutation mechanisms (CNV, promotor…)
  - Difficulties in recruiting large families…

Brain (1997), **120**, 479–490

Generalized epilepsy with febrile seizures plus
A genetic disorder with heterogeneous clinical phenotypes

Ingrid E. Scheffer and Samuel F. Berkovic

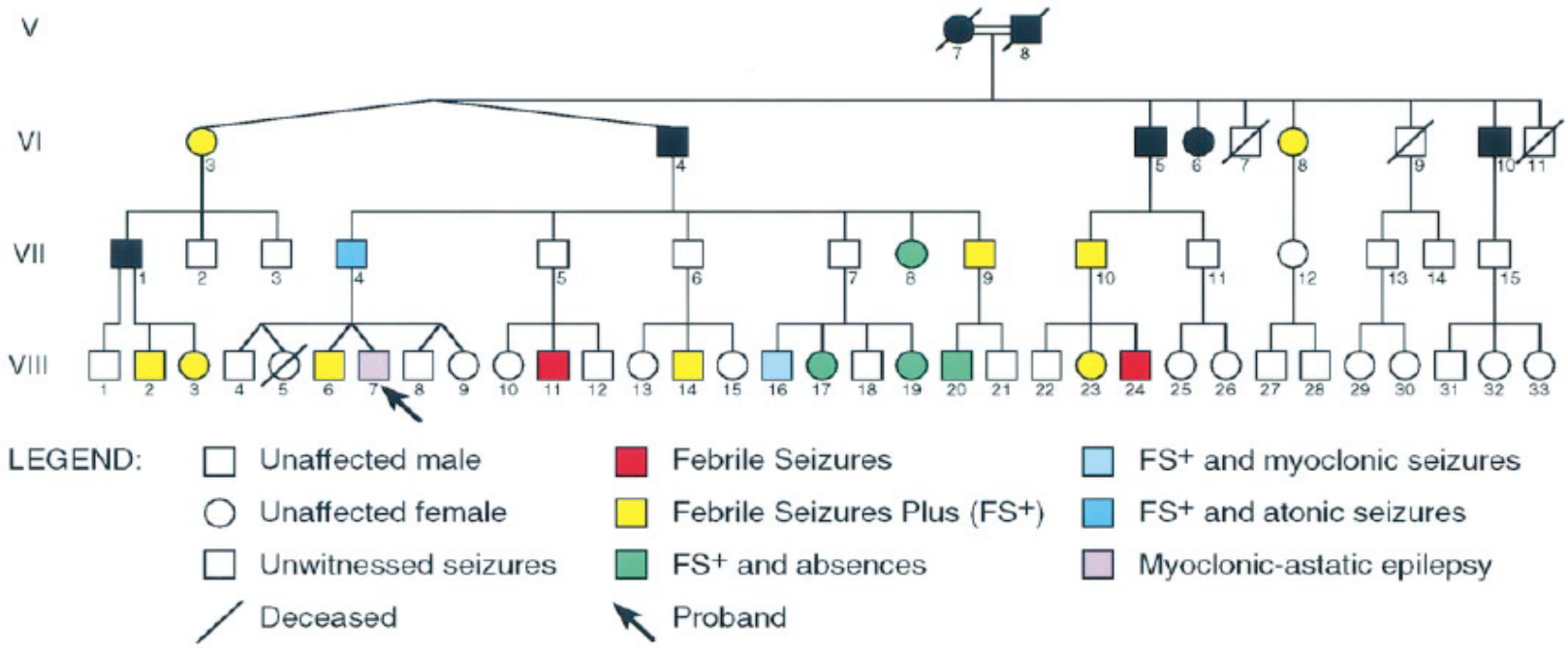
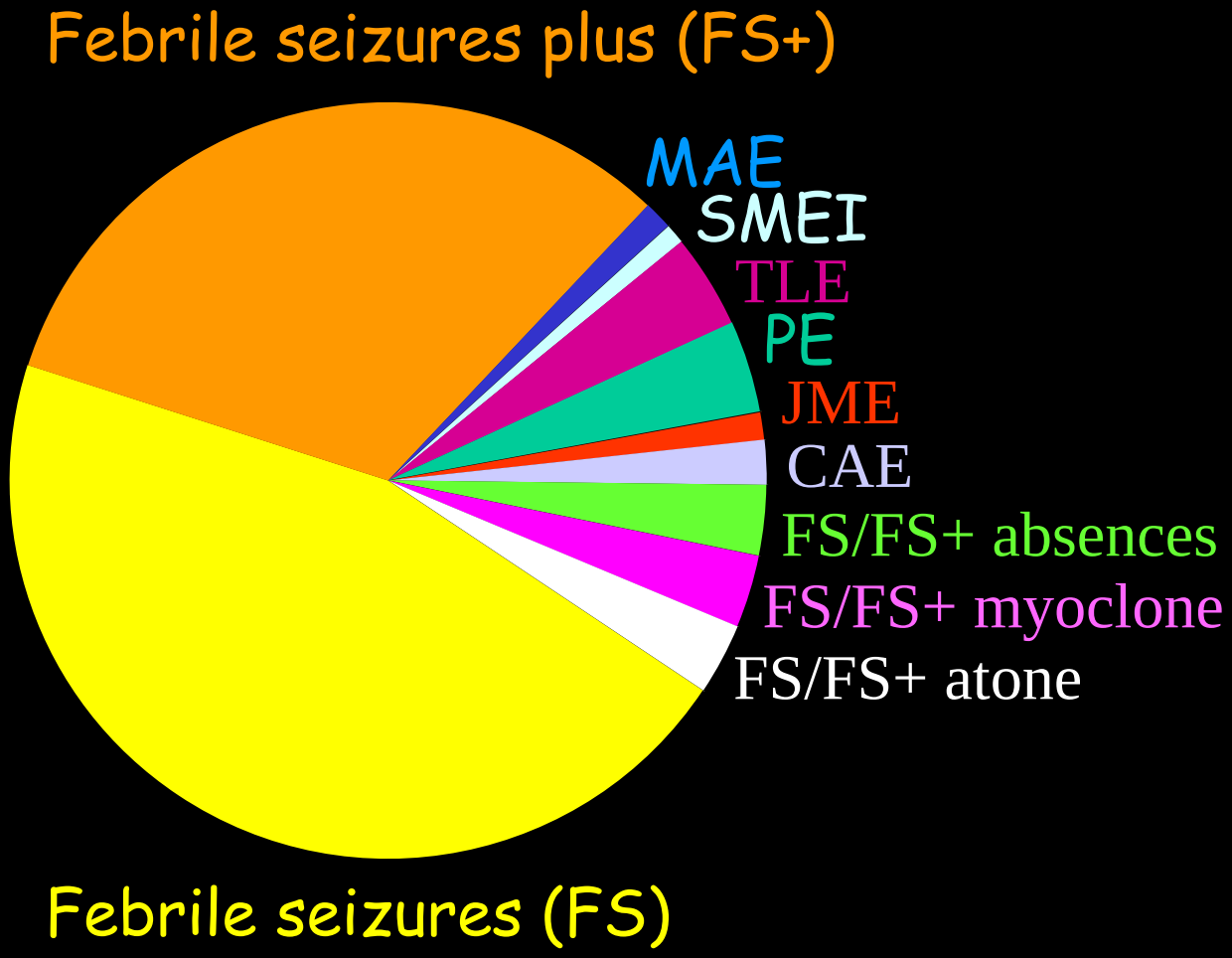


Fig. 4 Pedigree of the core family showing the heterogeneity of epilepsy phenotypes seen.

Generalized Epilepsy with Febrile Seizures Plus (GEFS+)



Human Genome project

- Start: 1990
- Completed: 2003
- Total cost: ~$3 billion

New tools

- Targeted next gen sequencing
  - "Epilepsy panels" e.g. 50 known "Epilepsy genes"
- Whole exome sequencing
  - 20.000 genes
- Whole genome sequening
- Genome-wide Copy Number Variant (CNV) analysis

Revolution in research and diagnostics

Exome Sequencing Allows for Rapid Gene Identification in a Charcot-Marie-Tooth Family

METHODS

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Massively Parallel Sequencing of Ataxia Genes after Array-Based Enrichment

Alexander Hoischen,[1*†] Christian Gilissen,[1†] Peer Arts,[1] Nienke Wieskamp,[1] Walter van der Vliet,[1] Sascha Vermeer,[1] Marloes Steehouwer,[1] Petra de Vries,[1] Rowdy Meijer,[1] Jorge Seiqueros,[2] Nine V.A.M. Knoers,[1] Michael F. Buckley,[1] Hans Scheffer,[1] and Joris A. Veltman[1]

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Whole-Genome Sequencing in a Patient with Charcot–Marie–Tooth Neuropathy

Analysis of Genetic Inheritance in a Family Quartet by Whole-Genome Sequencing

Jared C. Roach,[1*] Gustavo Glusman,[1*] Arian F. A. Smit,[1*] Chad D. Huff,[1,2*] Robert Hubley,[1] Paul T. Shannon,[1] Lee Rowen,[1] Krishna P. Pant,[3] Nathan Goodman,[1] Michael Bamshad,[4] Jay Shendure,[5] Radoje Drmanac,[3] Lynn B. Jorde,[2] Leroy Hood,[1†] David J. Galas[1†]

Genetic diagnosis by whole exome capture and massively parallel DNA sequencing

Murim Choi[a], Ute I. Scholl[a], Weizhen Ji[a], Tiewen Liu[a], Irina R. Tikhonova[b], Paul Zumbo[b], Ahmet Nayir[c], Ayşin Bakkaloğlu[d], Seza Özen[d], Sami Sanjad[e], Carol Nelson-Williams[a], Anita Farhi[a], Shrikant Mane[b], and Richard P. Lifton[a,1]

A *de novo* paradigm for mental retardation

Lisenka E L M Vissers[1,2], Joep de Ligt[1,2], Christian Gilissen[1], Irene Janssen[1], Marloes Steehouwer[1], Petra de Vries[1], Bart van Lier[1], Peer Arts[1], Nienke Wieskamp[1], Marisol del Rosario[1], Bregje W M van Bon[1], Alexander Hoischen[1], Bert B A de Vries[1], Han G Brunner[1,3] & Joris A Veltman[1,3]

VIB IB U

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  - For pushing the EuroEpinomics project forward
- "Kiel Group"
  - Ingo Helbig; Sarah Von Spiczak; Johanna Albers
- And …


ably wrong

Kick-off meeting RES CRP

- Antwerpen; Belgium
- 17th-18th November, 2011