Scuola Superiore di Neurologia

CORSO RESIDENZIALE SIN

Update su diagnosi e monitoraggio delle epilessie

Genova, 24 - 25 febbraio 2015

Accademia Nazionale di Medicina - Via M. Piaggio 17/6 - Genova

Analisi genetiche innovative nelle encefalopatie epilettiche

Marini Carla, MD, PhD

Neurologia Pediatrica

AUO Meyer

Firenze

OUTLINE

- Epileptic encephalopathies: definition
- Genetic epileptic encephalopathies
- Definition of the phenotype
  - Age at onset
  - Seizure types
  - Gender
  - EEG features
  - Pedigree
- Identification of candidate genes
- Genotype-phenotype correlation problems
- Diagnostic tools

Epileptic encephalopathy: definition

- Disorders in which seizures or paroxysmal interictal activity or both cause or contribute to progressive disturbance of cerebral function
  - impaired motor functions
  - cognitive delay or regression

- Epileptic encephalopathies represent about 40% of all epilepsies occurring in the first 3 years of life

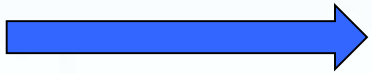
- may be due to structural abnormalities, either congenital or acquired, and **most often have a genetic etiology**

- OMIM recognizes 26 EIEE with mutations in specific genes http://www.ncbi.nlm.nih.gov/omim)

Monogenic Early Onset Epileptic Encephalopathies: OMIM

	gene	locus	Epilepsy/Syndrome
1	ARX	Xp22.13	Infantile spasms; Ohtahara Syndrome
2	CDKL5	Xp22	EOEE with spasms, focal & secondary generalized & myoclonic sz
3	SLC25A22	11p15.5	Neonatal epilepsy with suppression-burst, Early onser myclonic epilepsy
4	STXBP1	9q34.1	Infantile spasmsOhtahara Syndrome
5	SPTAN1	9q33-q34	Intractable sz with Hypsaritmia (2 Japanese pts) Sz with fever and severe MR (1 canadian pt)
6	SCN1A	2q24.3	Dravet syndrome
7	KCNQ2	20q13.33	Neonatal onset refractory sz
8	ARHGEF9	Xq11.1-q11.2	EOEE and iperreplexia
9	PCDH19	Xq22	Drug resistant focal or generalized sz, fever sensitivity and MR
10	PNKP	19q13.4	EOEE with microcefaly
11	SCNA2	2q24.3	EOEE with variable phenotype (4 Japanese pts)
12	PLCB1	20p12	EOEE (1 pt, homozygous mutation)
13	SCN8A	12q13.13	EOEE (1 pt)
14	KCNT1	9q34	Malignant migrating partial seizures of infancy (MMPSI)
15	ST3GAL3	1p34.1	EOEE: West Syndrome >Lennox-Gastaut (single palestinian family with consanguineity)
16	TBC1D24	16p13	EOEE: AR
17	GNAO1	16q13	EOEE: 4 unrelated girls
18	SZT2	1p34	EOEE: AR (homozygous or compaund heterozygous mutations)
19	GABRA1	5q34	EOEE: AD (heterozygous mutation
20	PIGA	Xp22.2	EOEE: X-linked recessive)
21	NECAP1	12p13.31	EOEE: AR (homozygous mutations)
22	SLC35A2	Xp11.23	EOEE: AD (hemizygous or heterozygous mutation)
23	DOCK7	1p31.3	EOEE: AR (compaund heterozygous mutations)
24	HCN1	5p12	EOEE: AD (heterozygous mutation)
25	SLC13A5	17p13.1	EOEE: AR (homozygous or compaund heterozygous mutations)
26	KCNB1	20q13.13	EOEE: AD heterozygous mutation

Phenotype



Genotype

- Age of seizure onset
- Seizure types
- EEG
- Gender
- Psychomotor development
- Additional features
- Pedigree
  - Single affected
  - Affected over multiple generations
  - Affected in a single generation
  - Consanguinity

- **KCNQ2**
- **KCNT1**
- **CDKL5**
- **STXBP1**
- **ARX**
- **SCN1A**
- **PCDH19**
- **GLUT1**

Age at seizure onset

- < 44 weeks of gestational age: **neonates**
- < 1 year: **infant**
- 1-12 years: **child**
- 12-18 years: **adolescent**
- > 18 years: **adult**

Seizure types

- Focal
- Spasms
- Clonic/hemiclonic
- Myoclonic
- Multifocal/migrating
- Tonic/symmetric or asymmetric/vibratory

EEG features

Interictal

- Suppression burst
- Focal paroxysmal activity
- Bilateral paroxysmal activity

Ictal: seizure recordings with clear cut definition of seizure type/s

Gender and development

- Some genetic epilepies have a predominant gender expression: PCDH19, CDKL5, MECP2, ARX

- Normal development before epilepsy onset followed by regression or lack of further acquisition
- Early psychomotor delay – prior to epilepsy onset
- Normal development before and after epilepsy

Additional features

- Dysmorphic features
- Other paroxysmal disorders
  - Migraine
  - Movement disorder
- Neuroimmaging:
  - Normal or non specific MRI
  - Malformation of cortical development

Family tree

Consistent with:

- Autosomal dominant inheritance
- Autosomal recessive inheritance
- Gender related
  - Only females affected
  - Only males affected

No additional affected family members:

- Reduced penetrance
- De novo mutations

Early onset epileptic encephalopathies

- Onset within the first few months or year of life
- Focal/multifocal drug resistant seizures
- Severe delay prior to seizures onset and/or worsened by seizures

KCNQ2 Encephalopathy: Emerging Phenotype of a Neonatal Epileptic Encephalopathy

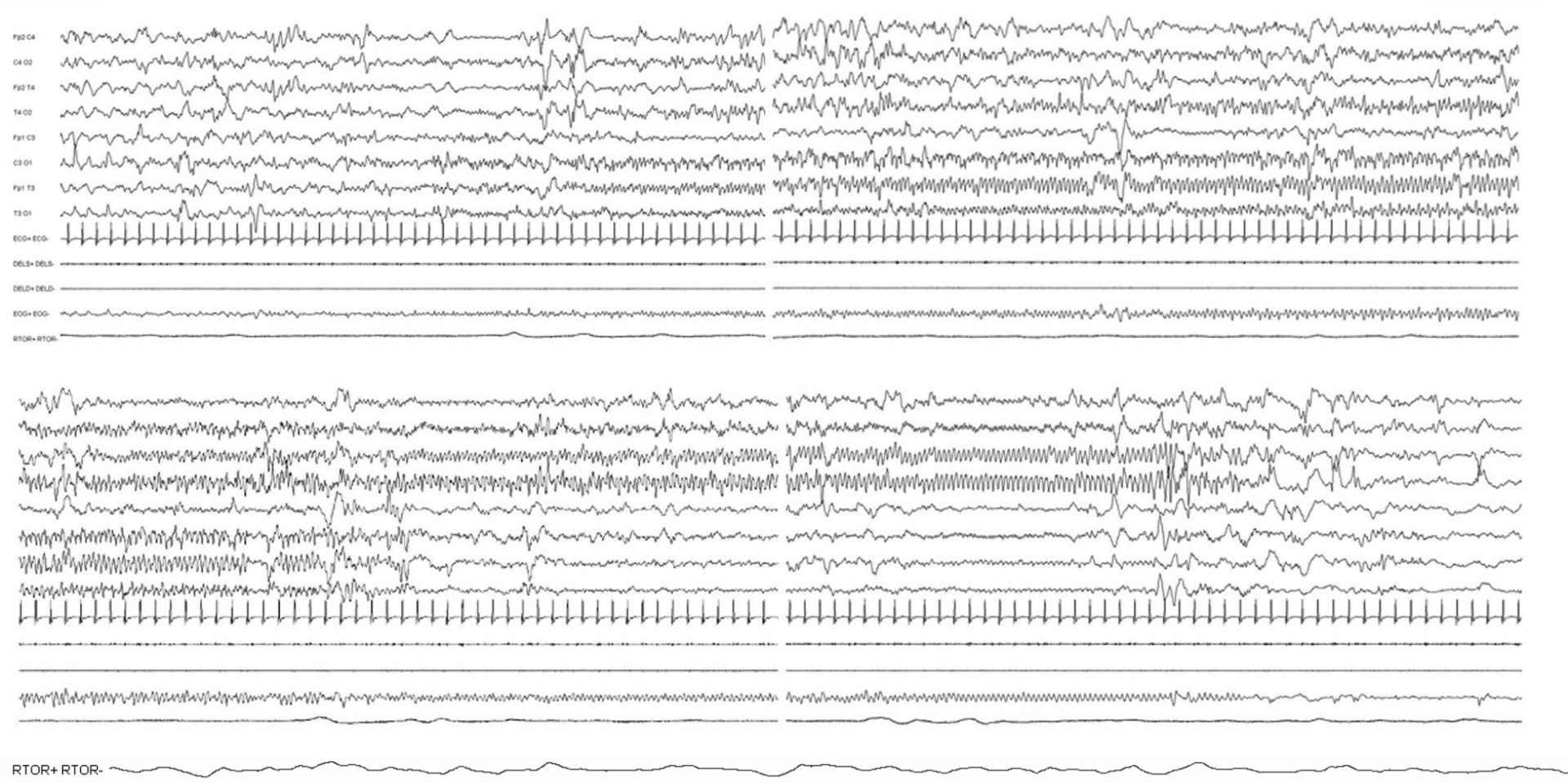
Sarah Weckhuysen, MD,[1,2,3] Simone Mandelstam, MB ChB,[4,5] Arvid Suls, PhD,[1,2] Dominique Audenaert, PhD,[1,2,6] Tine Deconinck, MSc,[1,2] Lieve R.F. Claes, PhD,[1,2] Liesbet Deprez, PhD,[1,2] Katrien Smets, MD,[1,2,7] Dimitrina Hristova, MD,[8] Iglika Yordanova, MSc,[9] Albena Jordanova, PhD,[1,2] Berten Ceulemans, MD, PhD,[2,10] An Jansen, MD, PhD,[11,12] Danièle Hasaerts, MD,[11] Filip Roelens, MD,[13] Lieven Lagae, MD, PhD,[14] Simone Yendle, BSc (Hons),[15] Thorsten Stanley, MD,[16] Sarah E. Heron, PhD,[17] John C. Mulley, PhD,[18,19] Samuel F. Berkovic, MD, FRS,[15] Ingrid E. Scheffer, MBBS, PhD,[4,15,20] and Peter de Jonghe, MD, PhD[1,2,7]

- Sz onset: first few weeks of life
- Sz type: focal with tonic component, apnea, cyanosis and prolonged bradycardia
- Sz generally resolve age age 3 yrs
- EEG: suppression burst/multifocal
- Intellectual disability with motor impairment
- MRI: early ‘transient’ basal ganglia and thalamus hyperintensities

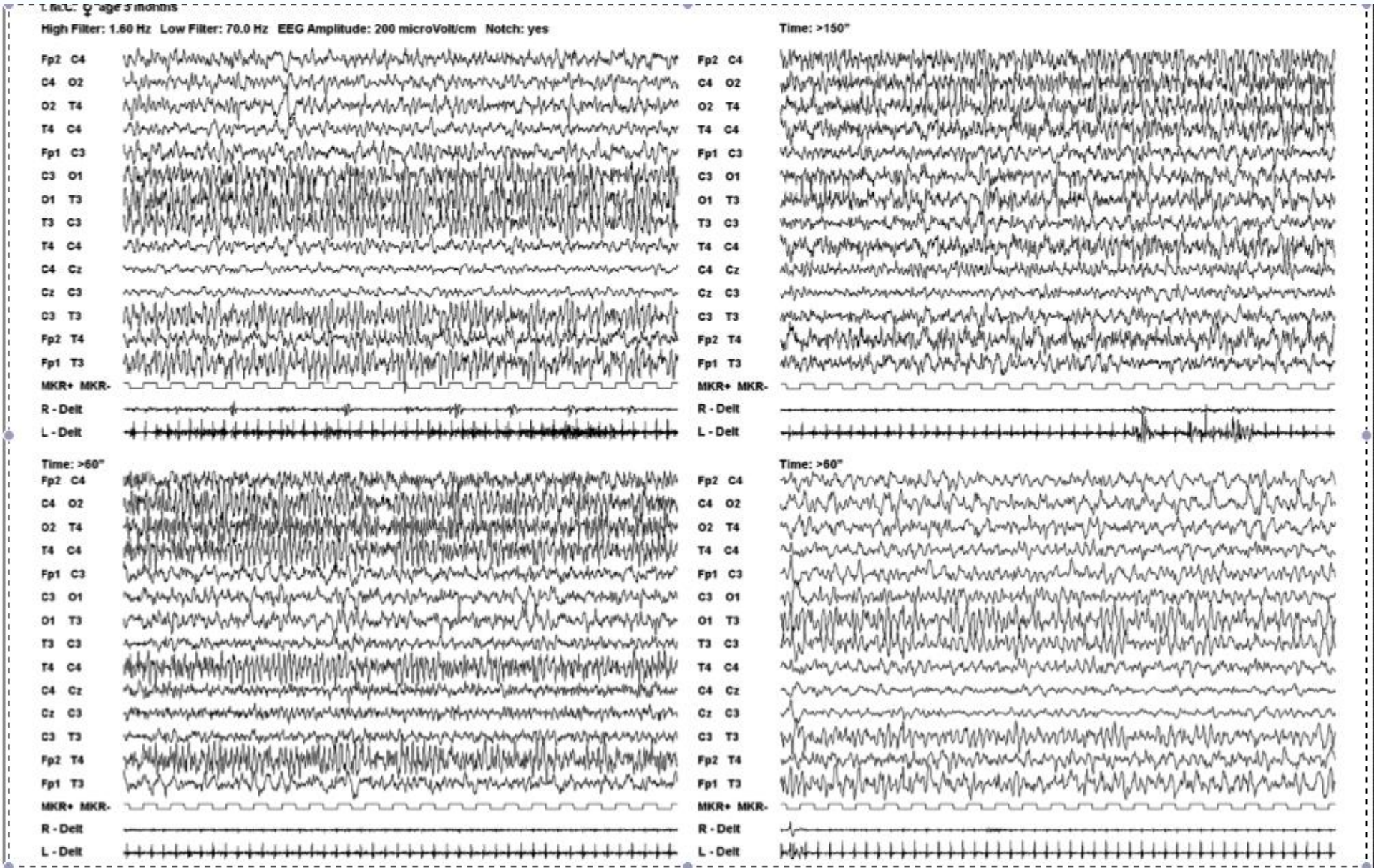
Ann Neurol. 2012;71(1):15-25

About 10% of unexplained EE beginning before the 1st month of life carry KCNQ2 abnormalities

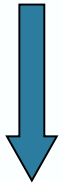
Good response to ev infusion of PTH and oral CBZ



Building up a phenotype:



Whole exome sequencing of 3 pts + parents



de novo variants in 2 unrelated probands….

De novo gain-of-function *KCNT1* channel mutations cause malignant migrating partial seizures of infancy

Giulia Barcia[1,2,12], Matthew R Fleming[3,4,12], Aline Deligniere[1], Valeswara-Rao Gazula[3], Maile R Brown[3], Maeva Langouet[5], Haijun Chen[6], Jack Kronengold[3], Avinash Abhyankar[7], Roberta Cilio[8], Patrick Nitschke[9], Anna Kaminska[10], Nathalie Boddaert[11], Jean-Laurent Casanova[7], Isabelle Desguerre[1], Arnold Munnich[5], Olivier Dulac[1,2], Leonard K Kaczmarek[3,4], Laurence Colleaux[5] & Rima Nabbout[1,2]

KCNT1: Na activated K channel regulating ion influx

Nature genetics 2012

Novel *SCN1A* Mutation in a Proband With Malignant Migrating Partial Seizures of Infancy

Emily R. Freilich, MD; Julie M. Jones, MS; William D. Gaillard, MD; Joan A. Conry, MD; Tammy N. Tsuchida, MD, PhD; Christine Reyes, MD; Sulayman Dib-Hajj, PhD; Stephen G. Waxman, MD; Miriam H. Meisler, PhD; Phillip L. Pearl, MD

Arch Neurol. 2011

De novo SCN1A mutations in migrating partial seizures of infancy

Carranza Rojo D, Hamiwka L, McMahon JM, Dibbens LM, Arsov T, Suls A, Stödberg T, Kelley K, Wirrell E, Appleton B, Mackay M, Freeman JL, Yendle SC, Berkovic SF, Bienvenu T, De Jonghe P, Thorburn DR, Mulley JC, Mefford HC, Scheffer IE.

Neurology. 2011;77(4):380-3

Building

- Onset fir
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- Very sev
- **EEG:**

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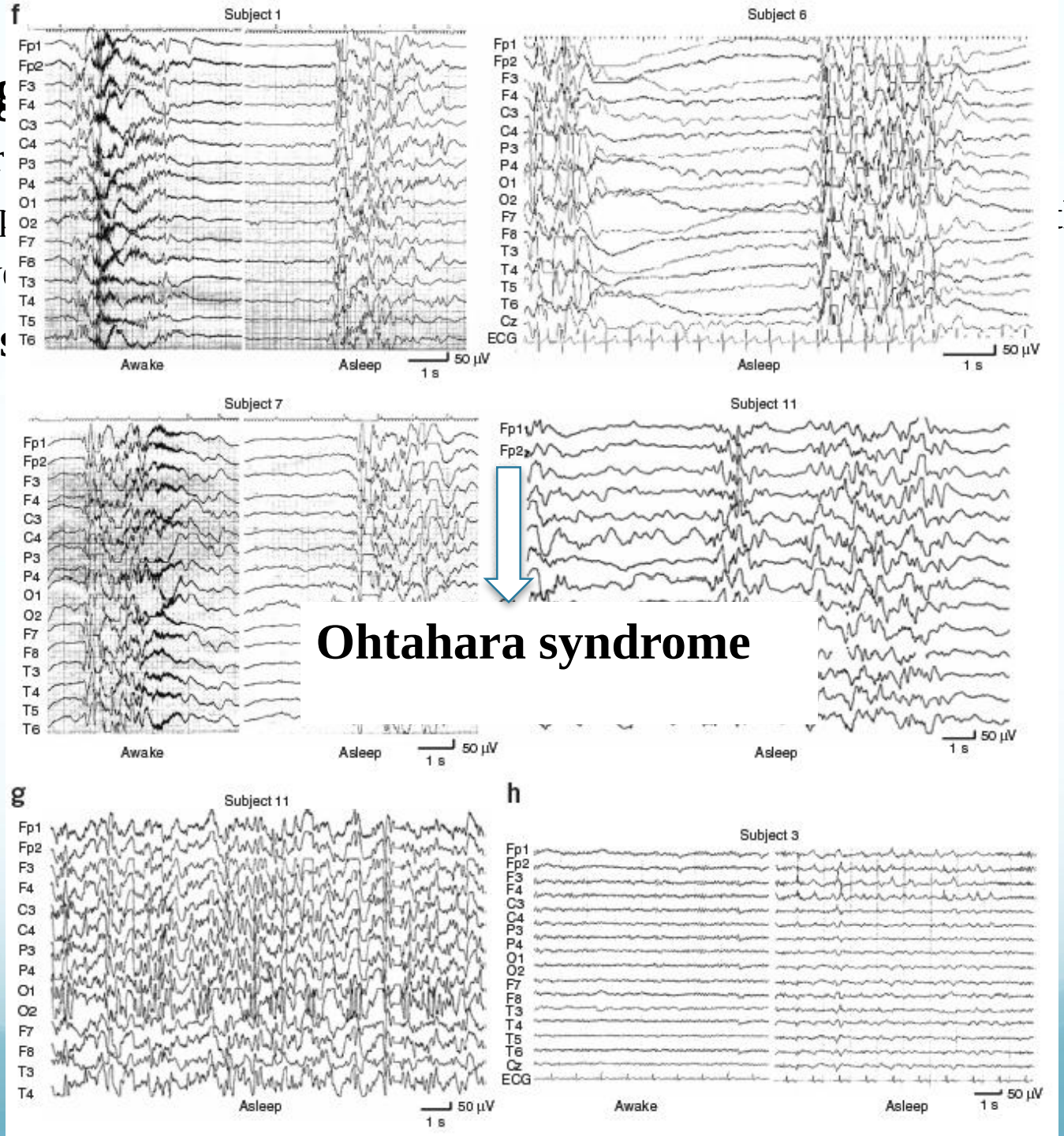


1

De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy

NATURE GENETICS VOLUME 40 | NUMBER 6 | JUNE 2008

Hirotomo Saitsu[1], Mitsuhiro Kato[2], Takeshi Mizuguchi[1], Keisuke Hamada[3], Hitoshi Osaka[4], Jun Tohyama[5], Katsuhisa Uruno[6], Satoko Kumada[7], Kiyomi Nishiyama[1], Akira Nishimura[1], Ippei Okada[1], Yukiko Yoshimura[1], Syu-ichi Hirai[8], Tatsuro Kumada[9], Kiyoshi Hayasaka[2], Atsuo Fukuda[9], Kazuhiro Ogata[3] & Naomichi Matsumoto[1]

2

Am J Hum Genet. 2007 Aug;81(2):361-6. Epub 2007 Jun 11.

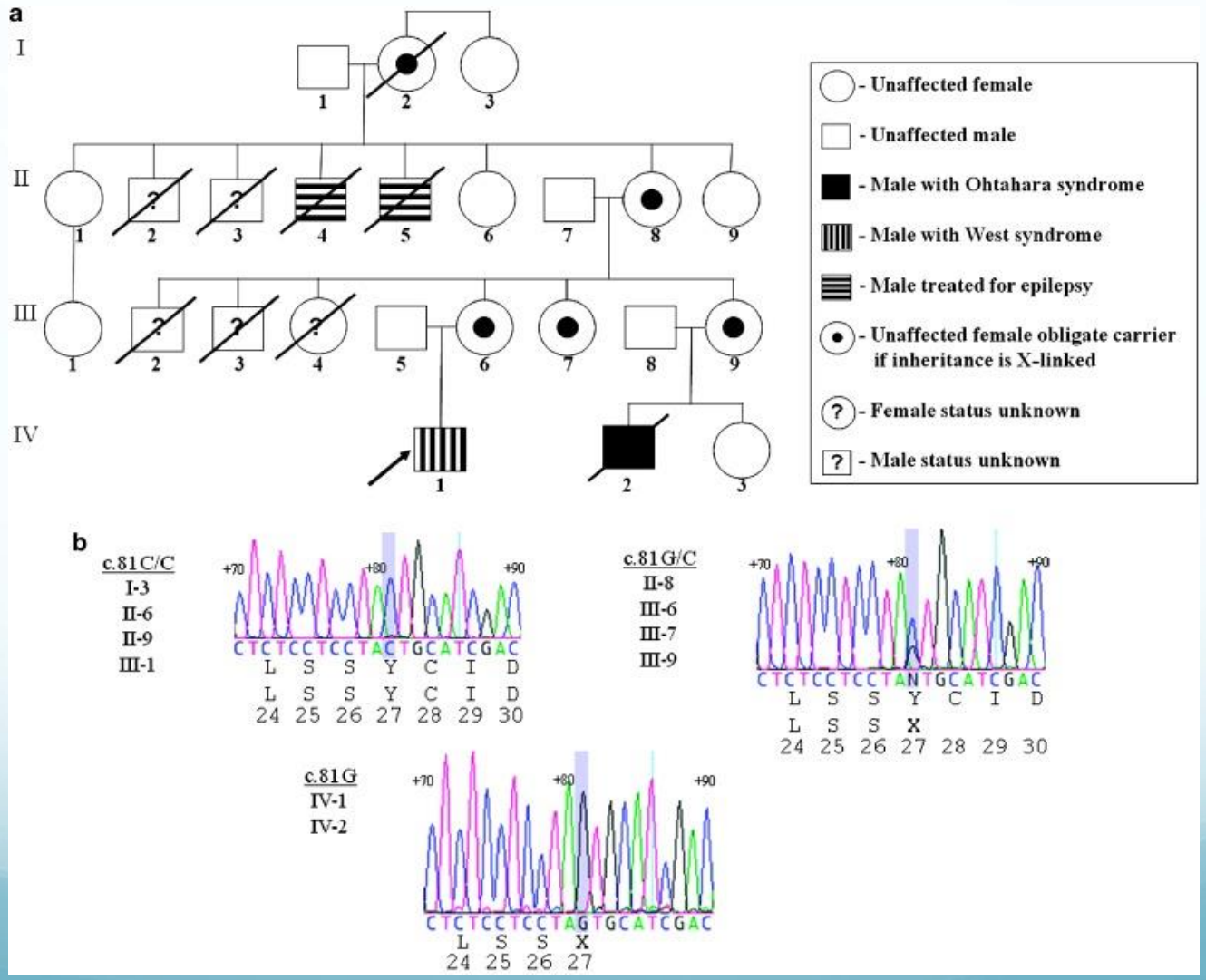
A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome).

Kato M[1], Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K.

Eur J Hum Genet. 2010 Feb;18(2):157-62. doi: 10.1038/ejhg.2009.139. Epub 2009 Sep 9.

Ohtahara syndrome in a family with an ARX protein truncation mutation (c.81C>G/p.Y27X).

Fullston T[1], Brueton L, Willis T, Philip S, MacPherson L, Finnis M, Gecz J, Morton J.



Building up a phenotype:

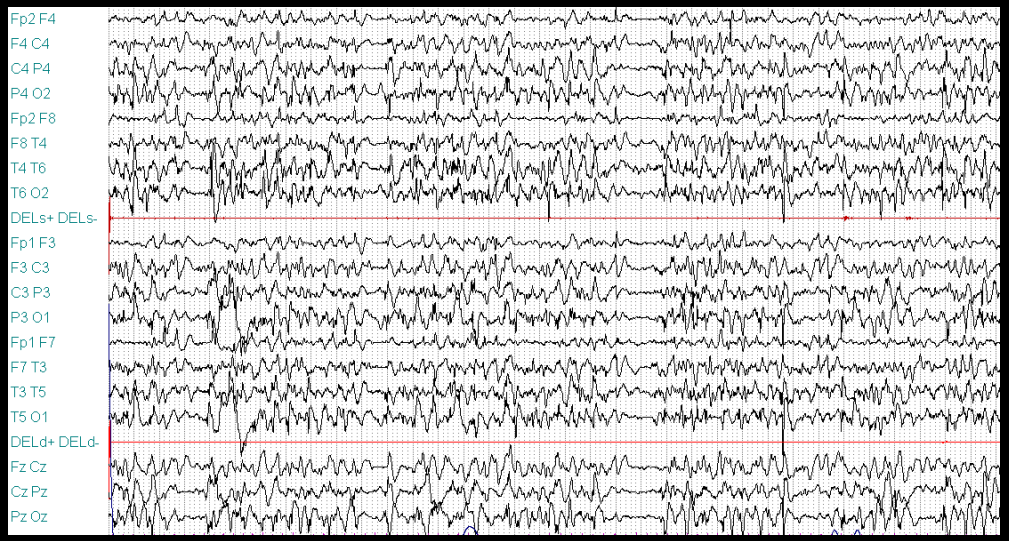
- Early infantile onset
- Seizure types
  - Tonic, clonic, spasms, myoclonic
- **Gender**
  - **F>>M**

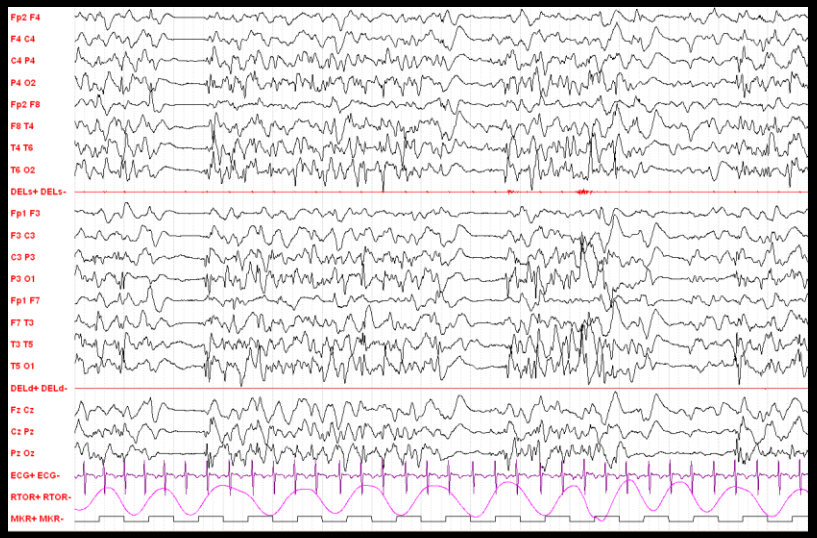
CDKL5 encephalopathy: emerging phenotype

- Early onset: 1-3 months of age
- F>>>M
- Focal or tonic-vibrating sz at onset
- Epileptic encephalopathy:
  - spasms and suppression burst
  - tonic
  - myoclonic
  - focal
- Severe delay with later ‘Rett like’ features
- Drug resistant epilepsy

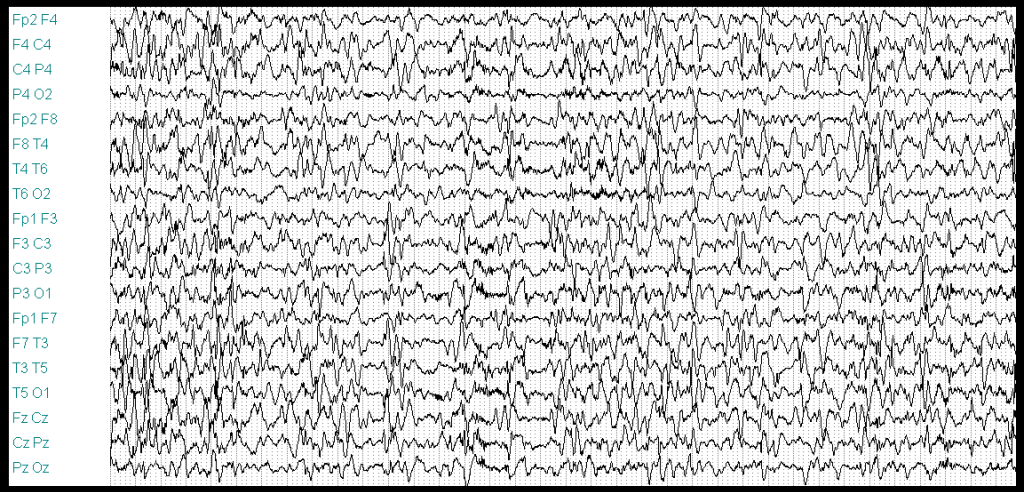
CDKL5: Xp22, CDKL5 and MeCP2 may belong to the same molecular pathway, neural maturation and synaptogenesis

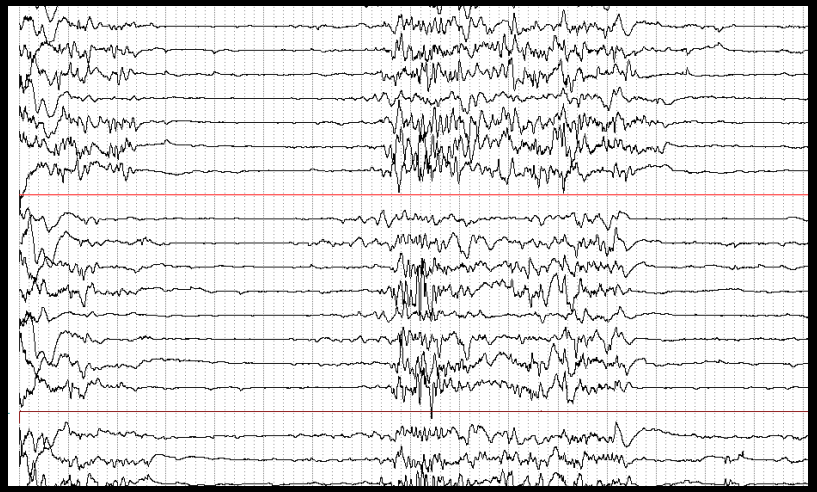
Interictal EEG: suppression-burst





- **K.M. 7m** **9m** **400 µV/cm**



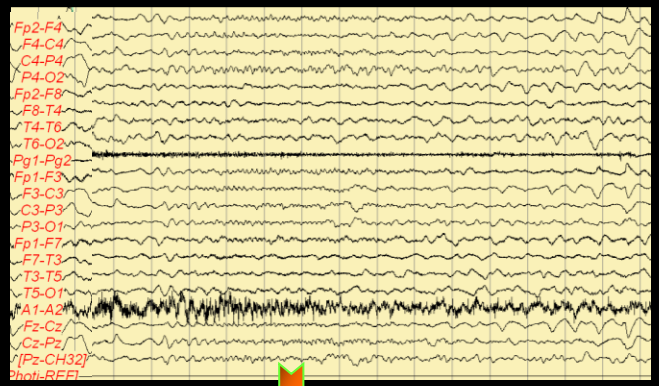


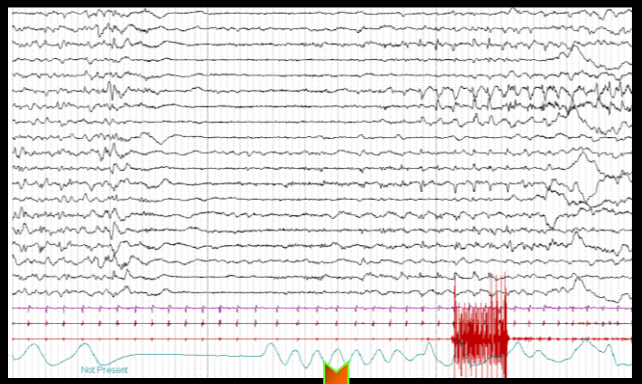
- **G.F. 4m** **7m** **300 µV/cm**

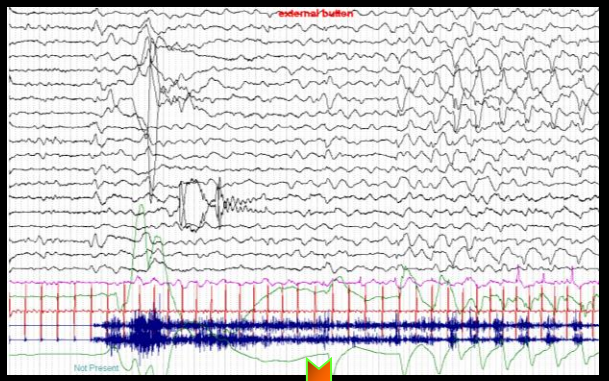
Melani et al. Dev Med Child Neurol. 2011;53(4):354-60

• tonic contraction, sustained spasm

SEIZURE START







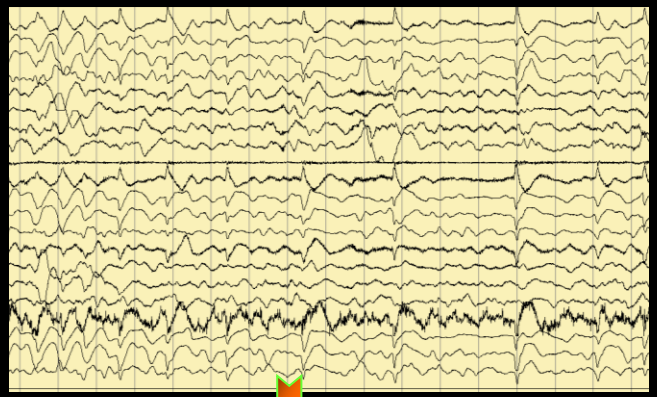
- PZ. 1

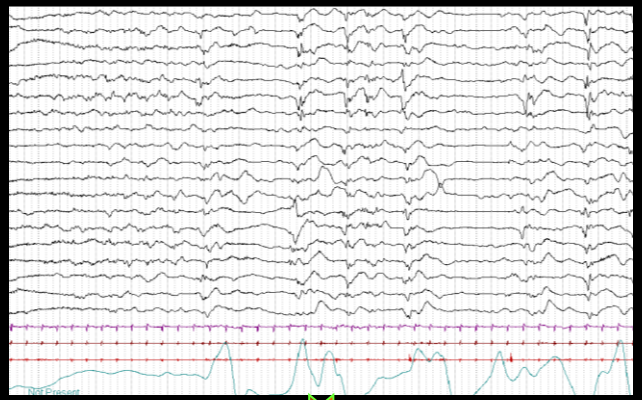
-PZ. 2

-PZ. 3

clusters of spasms/clonic sz

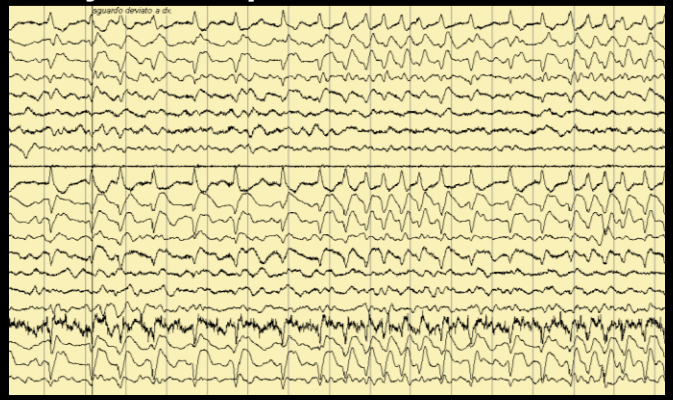
I° MIN

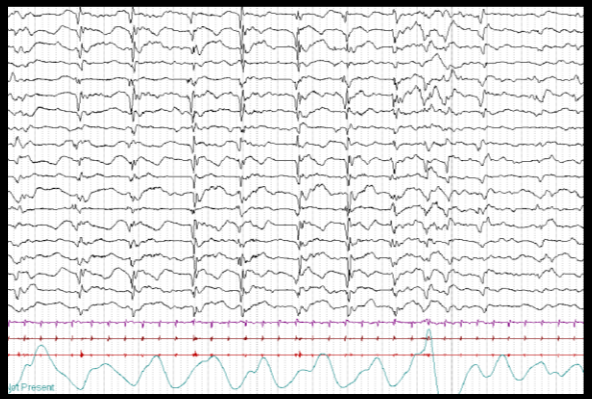


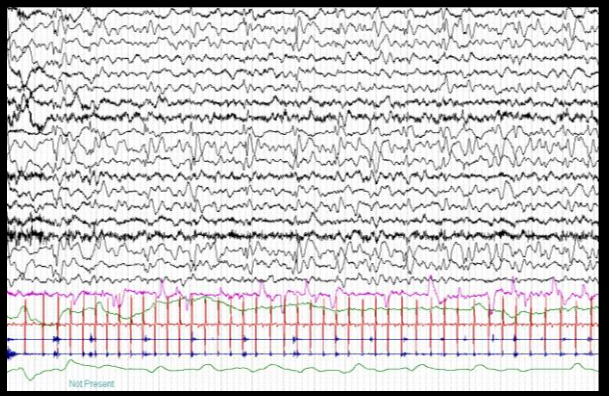


myoclonic phase

III° MIN







The three stages of epilepsy in patients with *CDKL5* mutations

*†Nadia Bahi-Buisson, †‡§Anna Kaminska, ¶#Nathalie Boddaert, ‖Marlène Rio, **Alexandra Afenjar, $Marion Gérard, ††Fabienne Giuliano, ‡‡‡Jacques Motte, §§Delphine Héron, ¶¶Marie Ange N'Guyen Morel, †‡§Perrine Plouin, ##Christian Richelme, ***Vincent des Portes, *†‡Olivier Dulac, †††Christophe Philippe, *†‡Catherine Chiron, *†‡Rima Nabbout, and ‡‡‡Thierry Bienvenu

- Stage I (1–10 wks): early epilepsy with normal interictal EEG frequent convulsive seizures
- Stage II (6 m-3 yrs): epileptic encephalopathy with infantile spasms and hypsarrhythmia
- Stage III (2.5-11 yrs): late multifocal and myoclonic epilepsy with tonic seizures and myoclonia

Epilepsia, 49(6):1027–1037, 2008

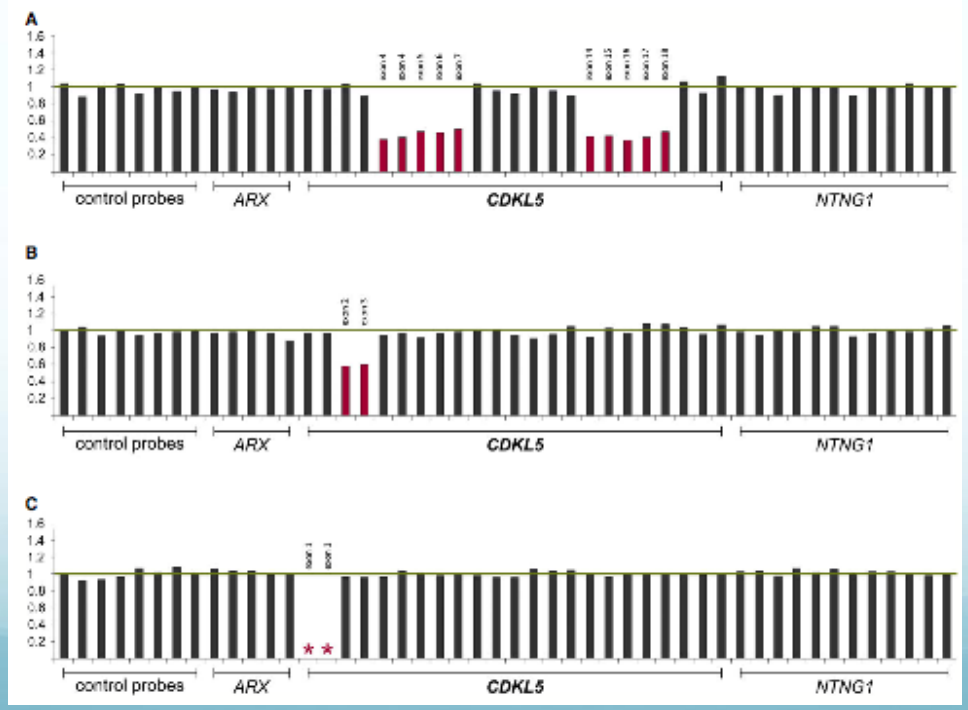
Optimizing the molecular diagnosis of *CDKL5* gene–related epileptic encephalopathy in boys

*Davide Mei, †Francesca Darra, *Carmen Barba, *Carla Marini, †Elena Fontana, *Laura Chiti, *Elena Parrini, †Bernardo Dalla Bernardina, and *Renzo Guerrini

Epilepsia 2015

Table 1. Clinical and genetic data of four boys harboring *CDKL5* mutations

Patient	Age at seizure onset/present age	Seizure type(s)°	Head circumference (°p) at birth/last follow-up	*CDKL5* gene abnormality (method, mutation, status)	Neurologic examination	EEG	MRI
1	2 m/15 y	Tonic, tonic–clonic	34 cm (50°p)/52 cm (−2 SD)	MLPA, deletion exons 4–7 and 14–18, mosaic	Spastic quadriparesis	Continuous slow spike-wave, electrodecremental events	Normal
2	2 m/7 y	Tonic, tonic–clonic, spasms	35 cm (50°p)/50.5 cm (25°p)	NGS, c.1449_1452dup p.Lys485Aspfs*11, mosaic	Spastic quadriparesis, hand stereotypies	Diffuse slow spike-wave, multifocal spikes	Normal
3	3 m/4 y	Tonic, focal, spasms	35 cm (50°p)/48 cm (−2 SD)	MLPA, deletion exons 2–3, mosaic	Spastic quadriparesis with dyskinesia, hand and head stereotypies	Hypsarrhythmia, multifocal spikes and spike-waves,	Mild cerebellar vermis hypoplasia
4	1 m/1 y	Tonic, spasms	35 cm (50°p)/45 cm (25°p)	MLPA and aCGH, deletion exon 1, full hemizygous	Quadriparesis with hypotonia, severely delayed milestones	Multifocal paroxysmal activity	Normal



Building up a phenotype:

- Infantile onset
  - clusters of spasms, often sleep-related
  - With or without hypsarrhythmia
  - Psychomotor developmental delay
- Gender
  - F=M
  - **M>>F**

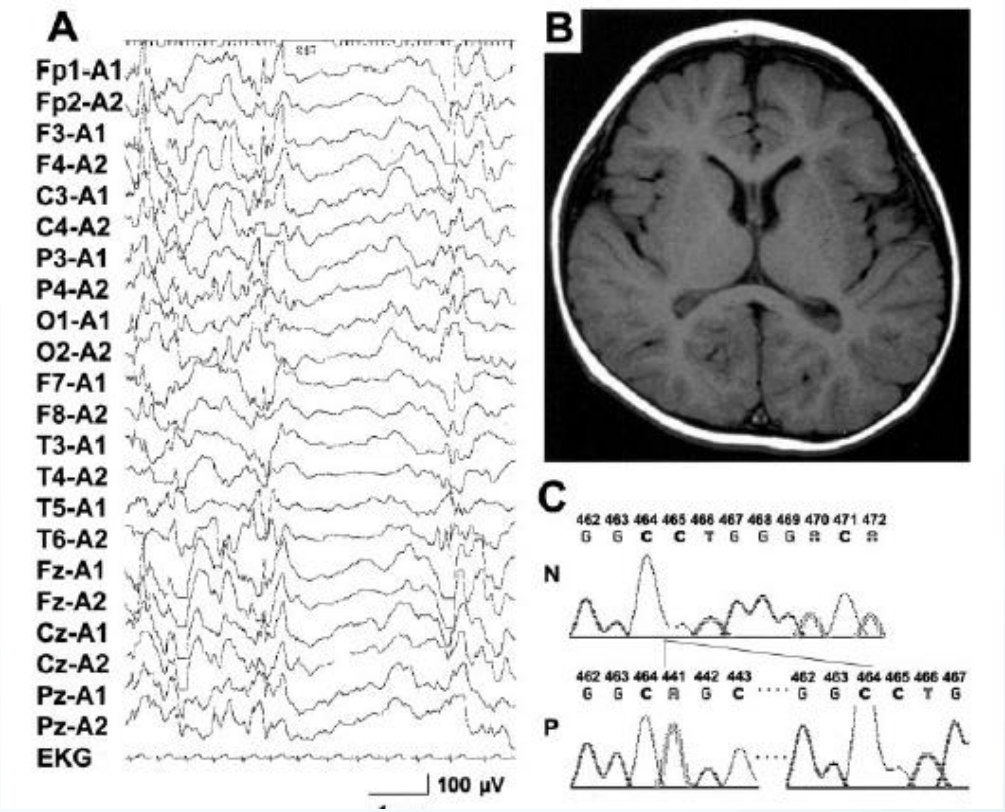
WEST syndrome and Infantile spasms

CME

Polyalanine expansion of *ARX* associated with cryptogenic West syndrome

M. Kato, MD, PhD; S. Das, PhD; K. Petras, BSc, Y. Sawaishi, MD, PhD; and W.B. Dobyns, MD

2003 NEUROLOGY



Epilepsia. 2010 Dec;51(12):2449-52. doi: 10.1111/j.1528-1167.2010.02767.x. Epub 2010 Nov 3.

STXBP1 mutations cause not only Ohtahara syndrome but also West syndrome--result of Japanese cohort study.

Otsuka M[1], Oguni H, Liang JS, Ikeda H, Imai K, Hirasawa K, Imai K, Tachikawa E, Shimojima K, Osawa M, Yamamoto T.

Hum Mol Genet. 2014 Sep 15;23(18):4846-58.

The genetic landscape of infantile spasms.

Michaud JL[1], Lachance M[2], Hamdan FF[2], Carmant L[1], Lortie A[1], Diadori P[1], Major P[1], Meijer IA[2], Lemyre E[3], Cossette P[4], Mefford HC[5], Rouleau GA[6], Rossigno E[7].

Author information

Abstract

Infantile spasms (IS) is an early-onset epileptic encephalopathy of unknown etiology in ~40% of patients. We hypothesized that unexplained IS cases represent a large collection of rare single-gene disorders. We investigated 44 children with unexplained IS using comparative genomic hybridisation arrays (aCGH) (n = 44) followed by targeted sequencing of 35 known epilepsy genes (n = 8) or whole-exome sequencing (WES) of familial trios (n = 18) to search for rare inherited or de novo mutations. aCGH analysis revealed de novo variants in 7% of patients (n = 3/44), including a distal 16p11.2 duplication, a 15q11.1q13.1 tetrasomy and a 2q21.3-q22.2 deletion. Furthermore, it identified a pathogenic maternally inherited Xp11.2 duplication. Targeted sequencing was informative for ARX (n = 1/14) and STXBP1 (n = 1/8). In contrast, sequencing of a panel of 35 known epileptic encephalopathy genes (n = 8) did not identify further mutations. Finally, WES (n = 18) was very informative, with an excess of de novo mutations identified in genes predicted to be involved in neurodevelopmental processes and/or known to be intolerant to functional variations. Several pathogenic mutations were identified, including de novo mutations in STXBP1, CASK and ALG13, as well as recessive mutations in PNPO and ADSL together explaining 28% of cases (5/18). In addition, WES identified 1-3 de novo variants in 64% of remaining probands, pointing to several interesting candidate genes. Our results indicate that IS are genetically heterogeneous with a major contribution of de novo mutations and that WES is significantly superior to targeted re-sequencing in identifying detrimental genetic variants involved in IS.

Clin Genet. 2008 Sep;74(3):288-90. doi: 10.1111/j.1399-0004.2008.01048.x. Epub 2008 Jun 28.

CDKL5 disruption by t(X;18) in a girl with West syndrome.

Nishimura A, Takano T, Mizuguchi T, Saitsu H, Takeuchi Y, Matsumoto N.

Building up a phenotype

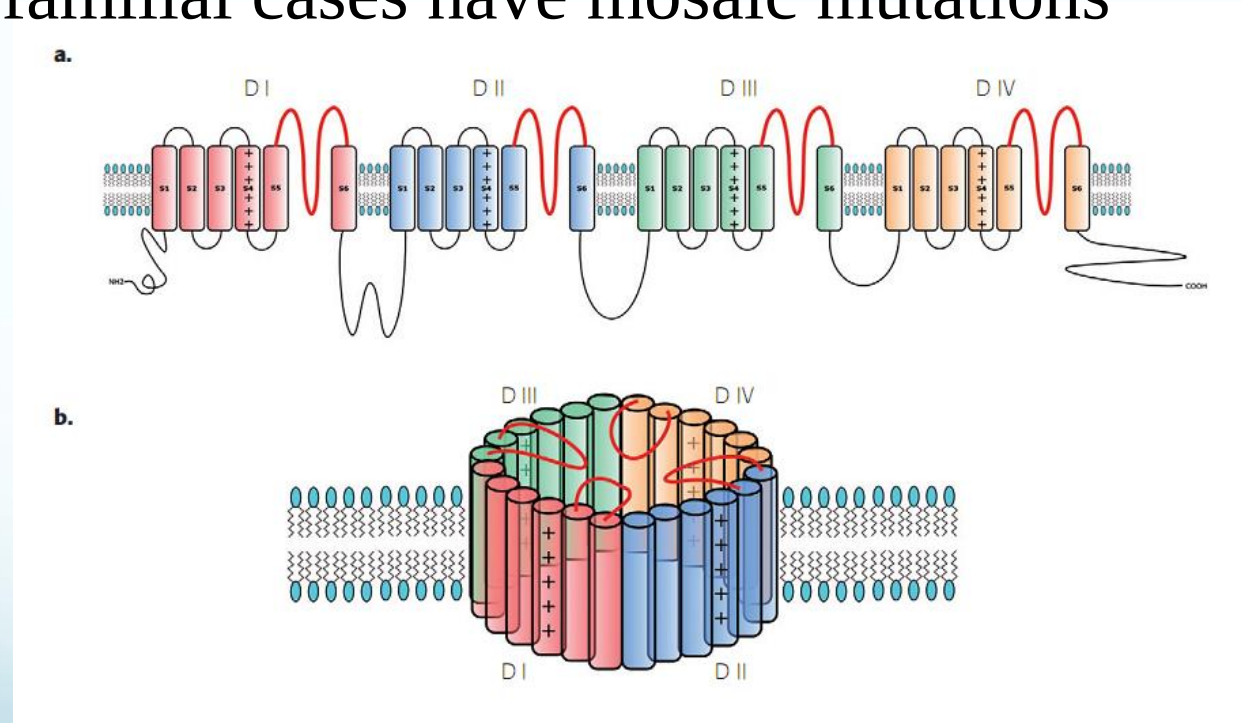
- Late infantile onset
- Seizure types
  - Hemiclonic, focal, myoclonic, focal
- Gender
  - F=M
- **Seizures related to fever** 

Severe myoclonic epilepsy of infancy (SMEI) or Dravet's syndrome

- SMEI: epileptic encephalopathy
- Onset: 1st year of life
- Seizure types
  - Febrile and afebrile seizures
  - Febrile or afebrile status epileptics
  - Myoclonic seizures
  - Absences
  - Focal seizures
- Cognitive impairment, usually moderate/severe, is correlated with the severity of the epilepsy
- Borderline clinical picture (SMEB)

Dravet syndrome

- ***SCN1A*** mutations in 70% of patients; 95% mut. de novo
- > 600 mutations identified; 10% CNVs involving *SCN1A* and or other contiguous genes
- 7% of familial cases have mosaic mutations



Most *SCN1A* mutation cause a persisten Na^+ current depolazization with subsequent neuronal hyperexcitability

Na^+ channels spectrum of phenotypes

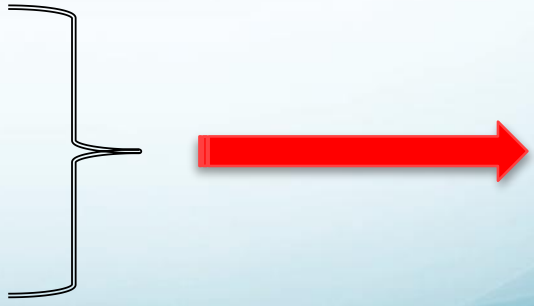
SCN1A abnormalities

- Dravet syndrome: 70-80%
- GEFS+: 10-15%
- MMPS: 13%

- FS, FS & TLE
- Panayiotopoulos syndrome
- Infantile spasms
- MAE
- Lennox-Gastaut
- Rasmussen encephalities
- Cryptogenic generalized epilepsy
- Cryptogenic focal epilepsy

Building up a phenotype

- Late infantile onset
- Seizure types
  - Hemiclonic, focal, myoclonic
- Gender
  - **Only females affected**
  - **Seizures related to fever**



X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment

Leanne M Dibbens[1,2,14], Patrick S Tarpey[3,14], Kim Hynes[1,4], Marta A Bayly[1], Ingrid E Scheffer[5,6], Raffaella Smith[3], Jamee Bomar[7], Edwina Sutton[4], Lucianne Vandeleur[1], Cheryl Shoubridge[1], Sarah Edkins[3], Samantha J Turner[5], Claire Stevens[3], Sarah O'Meara[3], Calli Tofts[3], Syd Barthorpe[3], Gemma Buck[3], Jennifer Cole[3], Kelly Halliday[3], David Jones[3], Rebecca Lee[3], Mark Madison[3], Tatiana Mironenko[3], Jennifer Varian[3], Sofie West[3], Sara Widaa[3], Paul Wray[3], John Teague[3], Ed Dicks[3], Adam Butler[3], Andrew Menzies[3], Andrew Jenkinson[3], Rebecca Shepherd[3], James F Gusella[8], Zaid Afawi[9], Aziz Mazarib[9], Miriam Y Neufeld[9], Sara Kivity[10], Dorit Lev[11], Tally Lerman-Sagie[11], Amos D Korczyn[8], Christopher P Derry[5], Grant R Sutherland[1,2,4], Kathryn Friend[1], Marie Shaw[1], Mark Corbett[1], Hyung-Goo Kim[8], Daniel H Geschwind[7], Paul Thomas[4], Eric Haan[1,4], Stephen Ryan[12], Shane McKee[13], Samuel F Berkovic[5], P Andrew Futreal[3], Michael R Stratton[3], John C Mulley[1,2,4] & Jozef Gécz[1,2,4]

Received 5 October 2007; accepted 11 March 2008; published online 11 May 2008;

nature genetics

- Epilepsy and mental retardation limited to females (EFMR)

- Focal and generalized seizures with onset in the 1st year of life

- *PCDH19:* atypical X-linked inheritance: only females are affected, males are healthy carriers

OPEN ACCESS Freely available online PLoS GENETICS

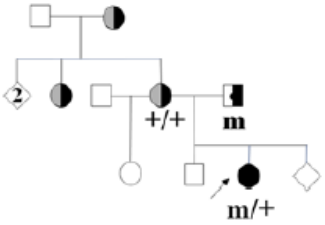
Sporadic Infantile Epileptic Encephalopathy Caused by Mutations in *PCDH19* Resembles Dravet Syndrome but Mainly Affects Females

PLoS Genet. 2009

Christel Depienne[1,2,3]*, Delphine Bouteiller[2], Boris Keren[1], Emmanuel Cheuret[4], Karine Poirier[5], Oriane Trouillard[1], Baya Benyahia[1], Chloé Quelin[5], Wassila Carpentier[6], Sophie Julia[4], Alexandra Afenjar[1,7], Agnès Gautier[8], François Rivier[9], Sophie Meyer[10], Patrick Berquin[11], Marie Hélias[12], Isabelle Py[13], Serge Rivera[14], Nadia Bahi-Buisson[15], Isabelle Gourfinkel-An[2,15,16], Cécile Cazeneuve[1], Merle Ruberg[2,3], Alexis Brice[1,2,3], Rima Nabbout[16,17], Eric LeGuern[1,2,3]

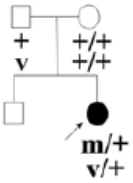
Family 4

m = p.Glu287X



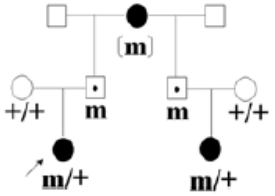
Family 5

m = p.Glu287X
v = p.Arg1107Gly



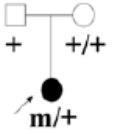
Family 6

m = c.1036_1040dup5



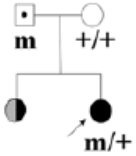
Family 7

m = c.506delC



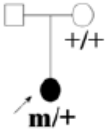
Family 8

m = p.Asp121Asn



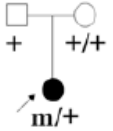
Family 9

m = p.Glu199Gln



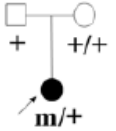
Family 10

m = p.Asn340Ser



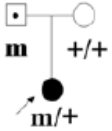
Family 11

m = p.Asn340Ser



Family 12

m = p.Leu543Pro



Focal seizures with affective symptoms are a major feature of *PCDH19*-gene-related epilepsy

Carla Marini[1], Francesca Darra[2], Nicola Specchio[3], Davide Mei[1], Alessandra Terracciano[4], Lucio Parmeggiani[5], Annarita Ferrari[6], Federico Sicca[6], Massimo Mastrangelo[7], Luigina Spaccini[7], Maria Lucia Canopoli[7], Elisabetta Cesaroni[8], Nelia Zamponi[8], Lorella Caffi[9], Paolo Ricciardelli[10], Salvatore Grosso[11], Tiziana Pisano[1], Maria Paola Canevini[12], Tiziana Granata[13], Patrizia Accorsi[14], Domenica Battaglia[15], Raffaella Cusmai[3], Federico Vigevano[3], Bernardo Dalla Bernardina[2] and Renzo Guerrini[1]

Epilepsia 2012

- 35 females with **unifocal or multifocal** seizures
- mean age of onset 10m
- "stormy" seizure onset, often related to fever
- Seizure severity does not clearly correlate with the cognitive deficit
- **Cognitive impairment is not always present:** 31% of our probands with focal epilepsy had normal cognitive functions
- Autistic features are frequent

From phenotype to genotype not always so straightforward

Same gene → different phenotypes

From benign ……

A Potassium Channel Mutation in Neonatal Human Epilepsy

Christian Biervert,* Björn C. Schroeder,* Christian Kubisch, Samuel F. Berkovic, Peter Propping, Thomas J. Jentsch,† Ortrud K. Steinlein†

SCIENCE • VOL. 279 • 16 JANUARY 1998

……. to epileptic encephalopathies

KCNQ2 Encephalopathy: Emerging Phenotype of a Neonatal Epileptic Encephalopathy

Sarah Weckhuysen, MD,[1,2,3] Simone Mandelstam, MB ChB,[4,5] Arvid Suls, PhD,[1,2] Dominique Audenaert, PhD,[1,2,6] Tine Deconinck, MSc,[1,2] Lieve R.F. Claes, PhD,[1,2] Liesbet Deprez, PhD,[1,2] Katrien Smets, MD,[1,2,7] Dimitrina Hristova, MD,[8] Iglika Yordanova, MSc,[9] Albena Jordanova, PhD,[1,2] Berten Ceulemans, MD, PhD,[2,10] An Jansen, MD, PhD,[11,12] Danièle Hasaerts, MD,[11] Filip Roelens, MD,[13] Lieven Lagae, MD, PhD,[14] Simone Yendle, BSc (Hons),[15] Thorsten Stanley, MD,[16] Sarah E. Heron, PhD,[17] John C. Mulley, PhD,[18,19] Samuel F. Berkovic, MD, FRS,[15] Ingrid E. Scheffer, MBBS, PhD,[4,15,20] and Peter de Jonghe, MD, PhD[1,2,7]

From phenotype to genotype not always so straightforward

Same gene → different phenotypes

From benign ……..

Benign Familial Neonatal-Infantile Seizures: Characterization of a New Sodium Channelopathy

Samuel F. Berkovic, MD,[1] Sarah E. Heron, BSc,[2] Lucio Giordano, MD,[3] Carla Marini, MD, PhD,[1,4] Renzo Guerrini, MD,[4] Robert E. Kaplan, MD,[5] Antonio Gambardella, MD,[6] Ortrud K. Steinlein, PhD,[7] Bronwyn E. Grinton, BSc,[1] Joanne T. Dean, BAppSc,[1] Laura Bordo, BSc,[8] Bree L. Hodgson, Dip Biomed Sci,[2] Toshiyuki Yamamoto, MD, PhD,[2] John C. Mulley, PhD,[2] Federico Zara, PhD,[8] and Ingrid E. Scheffer, MD, PhD[1]

to epileptic encephalopathy

Neurology. 2013 Sep 10;81(11):992-8. doi: 10.1212/WNL.0b013e3182a43e57. Epub 2013 Aug 9.

Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome.

Nakamura K[1], Kato M, Osaka H, Yamashita S, Nakagawa E, Haginoya K, Tohyama J, Okuda M, Wada T, Shimakawa S, Imai K, Takeshita S, Ishiwata H, Lev D, Lerman-Sagie T, Cervantes-Barragán DE, Villarroel CE, Ohfu M, Writzl K, Gnidovec Strazisar B, Hirabayashi S, Chitayat D, Myles Reid D, Nishiyama K, Kodera H, Nakashima M, Tsurusaki Y, Miyake N, Hayasaka K, Matsumoto N, Saitsu H.

From phenotype to genotype not always so straightforward

Same gene → different phenotypes

From epileptic encephalopathy

De novo gain-of-function *KCNT1* channel mutations cause malignant migrating partial seizures of infancy

Giulia Barcia[1,2,12], Matthew R Fleming[3,4,12], Aline Deligniere[1], Valeswara-Rao Gazula[3], Maile R Brown[3], Maeva Langouet[5], Haijun Chen[6], Jack Kronengold[3], Avinash Abhyankar[7], Roberta Cilio[8], Patrick Nitschke[9], Anna Kaminska[10], Nathalie Boddaert[11], Jean-Laurent Casanova[7], Isabelle Desguerre[1], Arnold Munnich[5], Olivier Dulac[1,2], Leonard K Kaczmarek[3,4], Laurence Colleaux[5] & Rima Nabbout[1,2]

Nat Genet 2012

To…….focal epilepsy

Nat Genet. 2012 Nov;44(11):1188-90. doi: 10.1038/ng.2440. Epub 2012 Oct 21.

Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy.

Heron SE[1], Smith KR, Bahlo M, Nobili L, Kahana E, Licchetta L, Oliver KL, Mazarib A, Afawi Z, Korczyn A, Plazzi G, Petrou S, Berkovic SF, Scheffer IE, Dibbens LM.

From phenotype to genotype not always so straightforward

Same phenotype  different genes

West syndrome/Infantile spasms

ARX
STXBP1
CDKL5
FOXG1
SPTAN1

From phenotype to genotype not always so straightforward

Same gene → different phenotypes

Phenotypes and genotypes associated with ARX mutations

	Phenotype (gender)	*ARX* genotypes
Syndromes with malformations	XLAG with HYD (M)	
	XLAG (M)	
	Proud syndrome (ACC-AG) (M)	Large intragenic deletions, frameshifts or null mutations (exons 1–4), nonconservative missense mutations in homeobox
	ACC with MR, seizures (F)	
	ACC with normal intelligence (F)	
Syndromes without malformations	Infantile epileptic–dyskinetic encephalopathy (this report) (M)	PolyA expansion (1st PolyA tract [GCG]7)
	Infantile spasms (M)	PolyA expansion (1st [GCG]7 and 2nd PolyA tracts), deletion of exon 5
	XMESID (M)	Rarely, conservative missense mutations in homeobox
	Partington syndrome (XLMR, seizures, mild distal dystonia) (M)	PolyA expansion (2nd PolyA tract)
	XLMR with or without seizures (M)	PolyA expansion (1st [GCG]1, 2, 3 and 2nd PolyA tracts), missense mutations outside homeobox
	Normal (F)	PolyA expansion, missense mutation

STXBP1: spectrum of phenotypes

At present about 30 known mutations:

1) Ohtahara syndrome: about 10% *(Milh et al, epilepsia 2011)*

2) West Syndrome: 3 patients (*Deprez et al, 2010, Otsuka et al. 2010*)

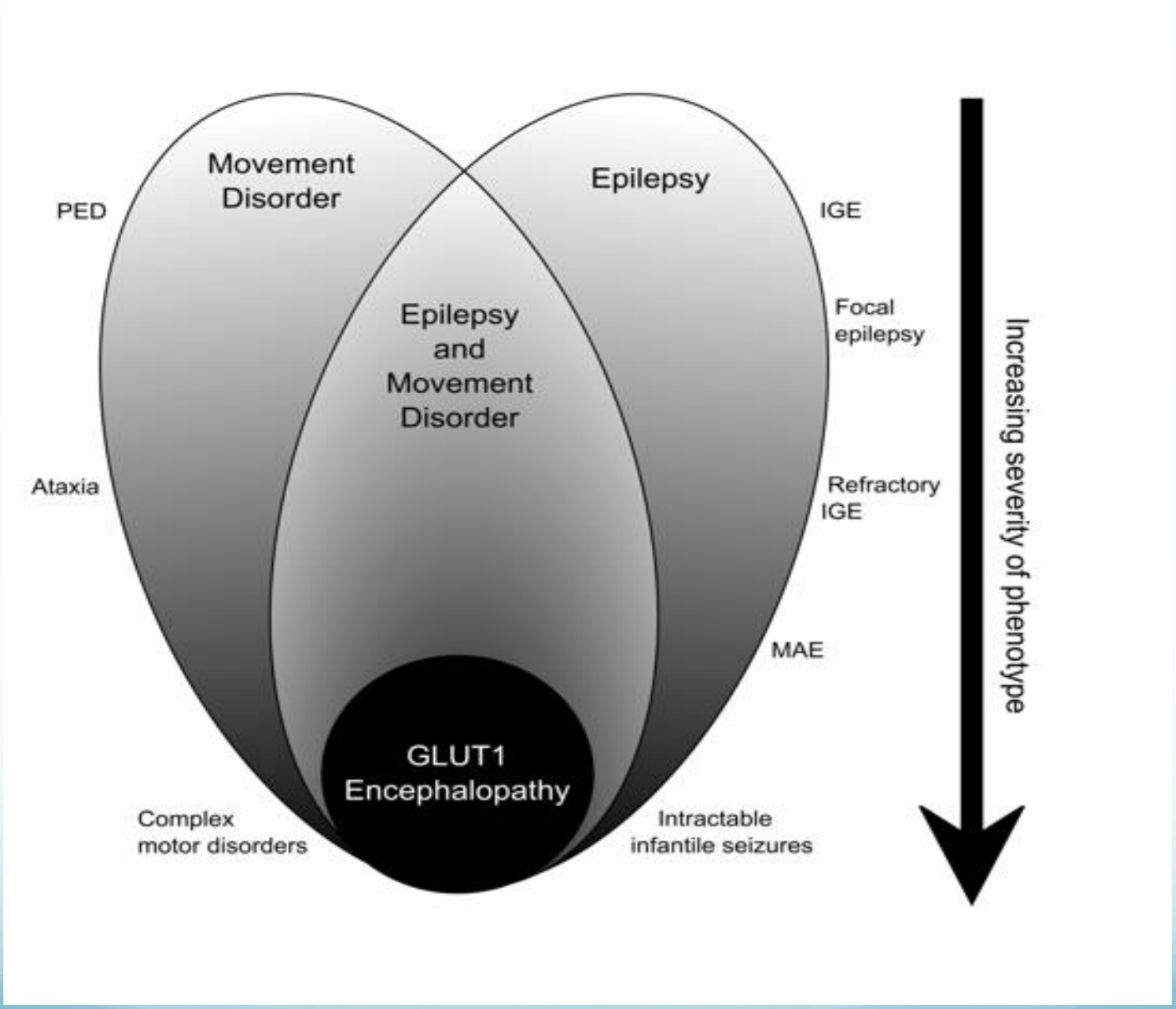
3) Other ‘less defined’ EOEE: few patients (Hamdan et al 2009, *Deprez et al, 2010*)

4) Infantile spasms, focal seizures, intellectual disability and generalized tremor: 3 patients (*Mignot et al, 2011*)

5) Intellectual disabiliy without epilepsy (*Hamdan et al, 2011*)

GLUT1 deficiency syndrome

- GLUT1 deficiency syndrome (GLUT1DS) is a **treatable epileptic encephalopathy** caused by impaired glucose uptake at the blood–brain barrier and into brain cells
- Patients present with early-onset epilepsy, developmental delay, acquired microcephaly and complex movement disorders
- Phenotype is highly variable and several atypical variants have been described
- The condition is diagnosed by
  - hypoglycorrhachia
  - heterozygous mutations in *SLC2A1* gene on chr. 1p35



Mullen et al 2011

The new ‘era’ of whole exome sequencing (WES)

- New EOEE genes
- WES in trios
- WES in families
- Panels of genes
- Large consortium studies
- ? Interpreting the WES data

De novo mutations in epileptic encephalopathies

Epi4K Consortium* & Epilepsy Phenome/Genome Project*

Exome sequencing of 264 probands and their parents

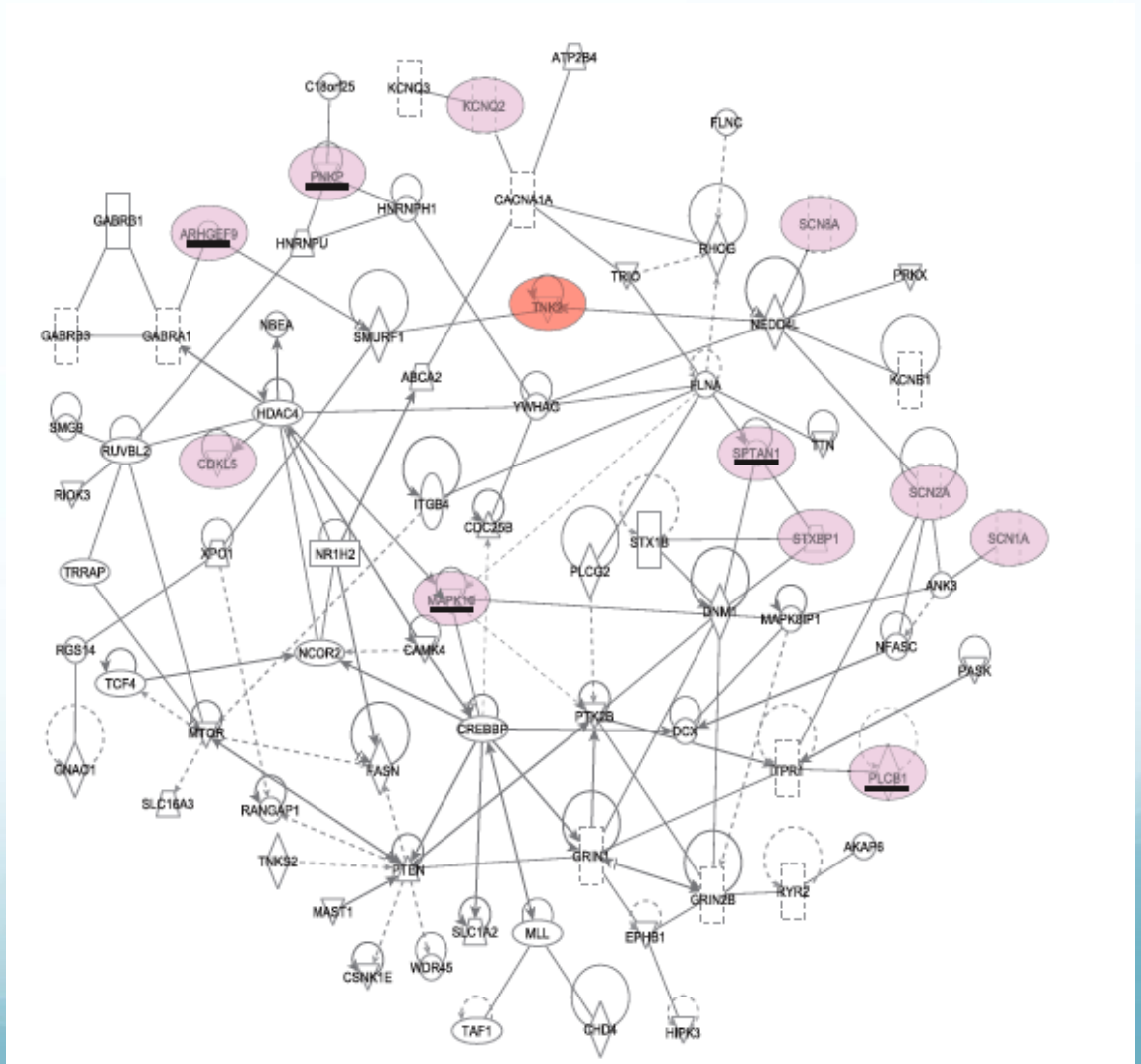
-West Syndrome

- Lennox-Gastaut

329 de novo mutations

NO common genes for similar phenotypes

Private mutations



De Novo Loss-of-Function Mutations in *CHD2* Cause a Fever-Sensitive Myoclonic Epileptic Encephalopathy Sharing Features with Dravet Syndrome

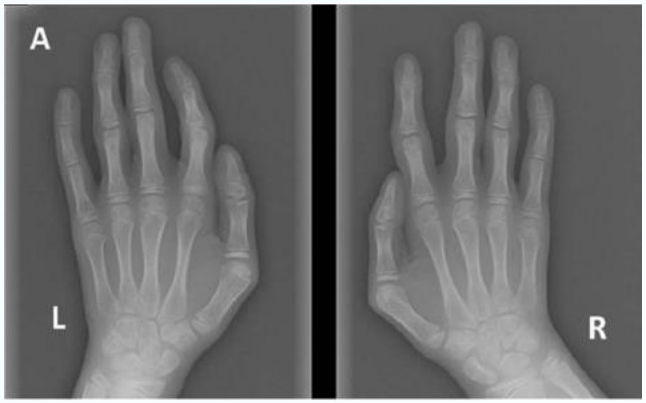
Arvid Suls,[1,2,38] Johanna A. Jaehn,[3,38] Angela Kecskés,[4,38] Yvonne Weber,[5,38] Sarah Weckhuysen,[1,2] Dana C. Craiu,[6,7] Aleksandra Siekierska,[4] Tania Djémié,[1,2] Tatiana Afrikanova,[4] Padhraig Gormley,[8] Sarah von Spiczak,[3] Gerhard Kluger,[9] Catrinel M. Iliescu,[6,7] Tiina Talvik,[10,11] Inga Talvik,[10,11] Cihan Meral,[12] Hande S. Caglayan,[13] Beatriz G. Giraldez,[14] José Serratosa,[14] Johannes R. Lemke,[15] Dorota Hoffman-Zacharska,[16] Elzbieta Szczepanik,[17] Nina Barisic,[18] Vladimir Komarek,[19] Helle Hjalgrim,[20,21] Rikke S. Møller,[20] Tarja Linnankivi,[22] Petia Dimova,[23] Pasquale Striano,[24] Federico Zara,[25] Carla Marini,[26] Renzo Guerrini,[26] Christel Depienne,[27,28,30] Stéphanie Baulac,[27,28,29] Gregor Kuhlenbäumer,[31] Alexander D. Crawford,[4,32] Anna-Elina Lehesjoki,[33,34,35] Peter A.M. de Witte,[4] Aarno Palotie,[8,36,37] Holger Lerche,[5] Camila V. Esguerra,[4,39] Peter De Jonghe,[1,2,39,*] Ingo Helbig,[3,39] and the EuroEPINOMICS RES Consortium

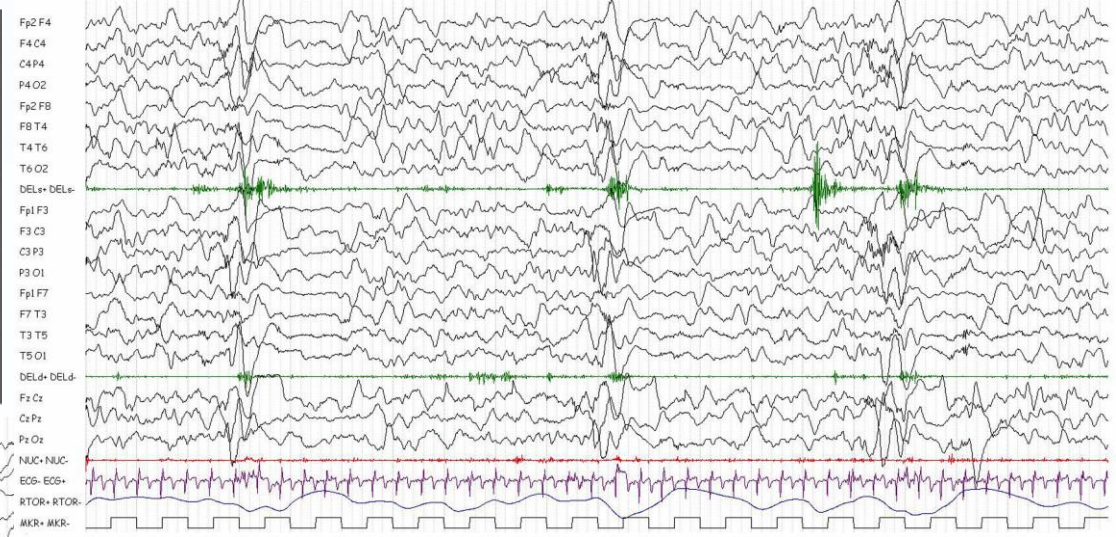
9 SCN1A-negative DS underwent WES

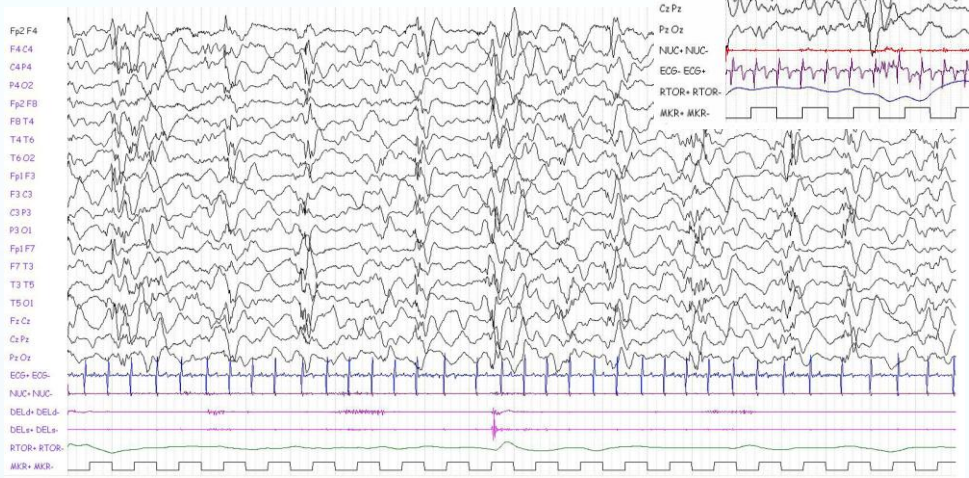
3/9 carried CHD2 mutations

- infantile onset
- Fever-related generalized seizures
- Myoclonic seizures
- Atypical absences, atonic seizures
- Cognitive impairment

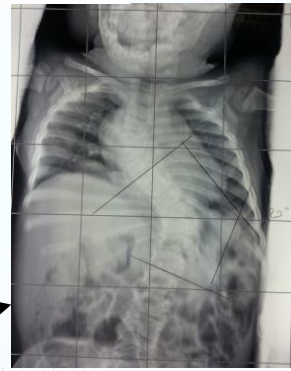
The American Journal of Human Genetics *93*, 967–975, November 7, 2013

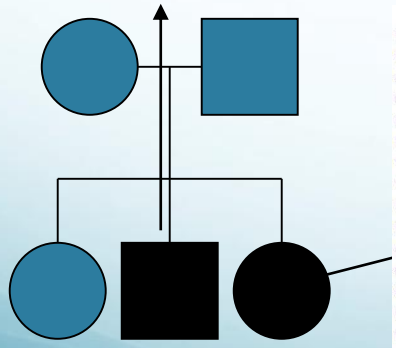


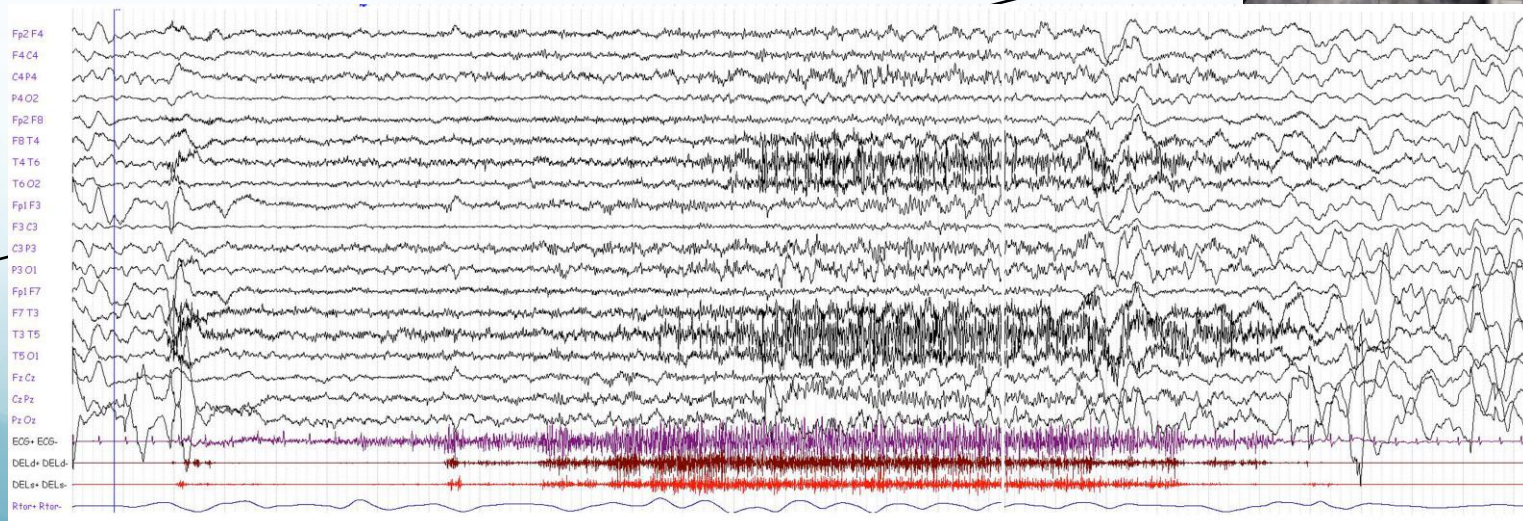




First few mths of life: spasms and tonic-vibratory sz







Whole exome sequencing of the quartet showed:

SLC35A3 compound heterozygous mutations in both siblings, both parents carried a single heterozygous mutation

SLC35A3: chr 1p21; 9 exons

transporter of the UDP-N-acetylglucosamine (UDP-GlcNAc) from its site of synthesis in the cytosol to its site of use in the Golgi

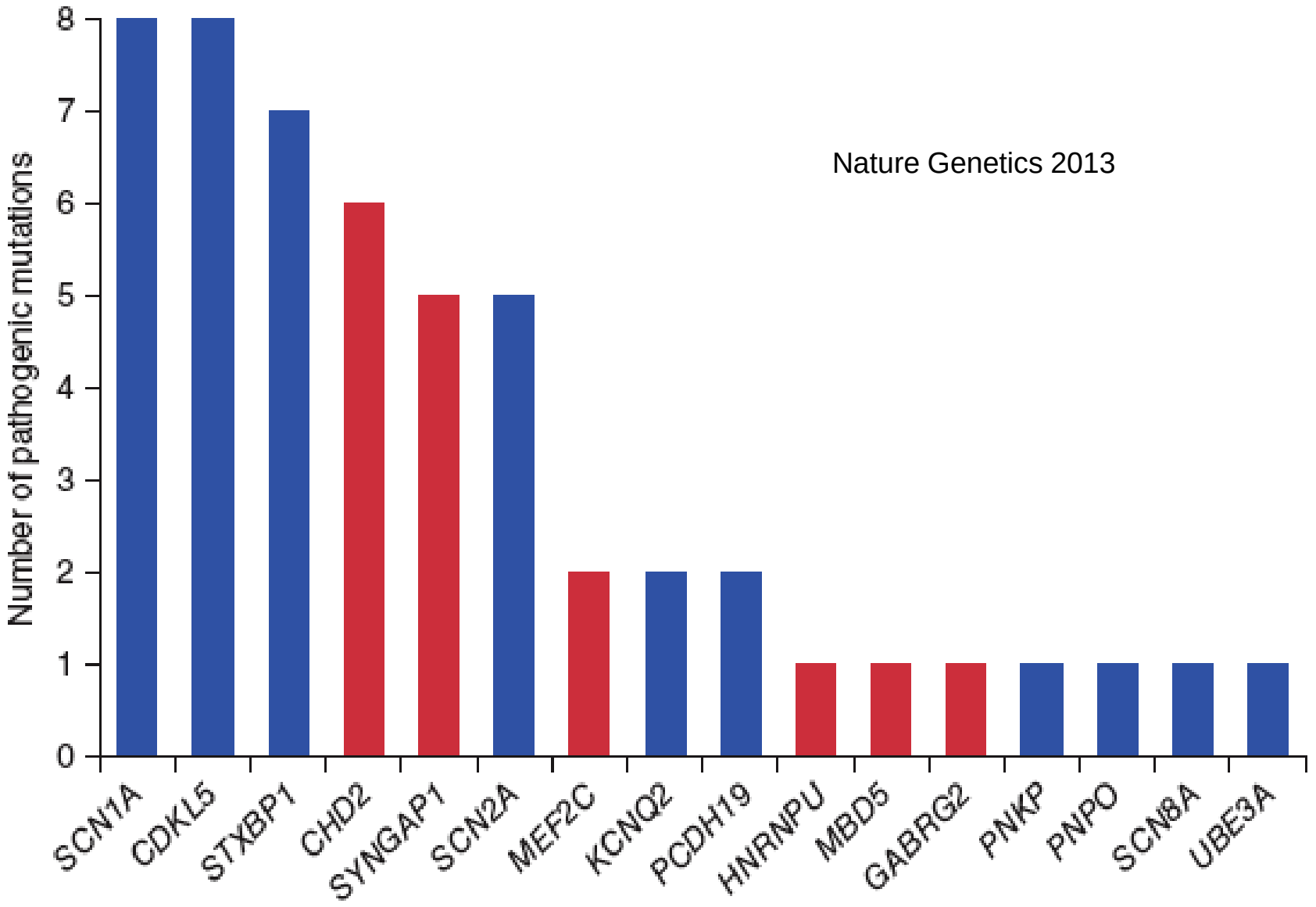
Proper function of the transporter is essential for byosintheis of glicoproteins, glucolipids and proteoglycans

Mutations in SLC35A3 cause autism spectrum disorder, epilepsy and arthrogryposis

Simon Edvardson, Angel Ashikov, Chaim Jalas, et al.

J Med Genet published online September 12, 2013

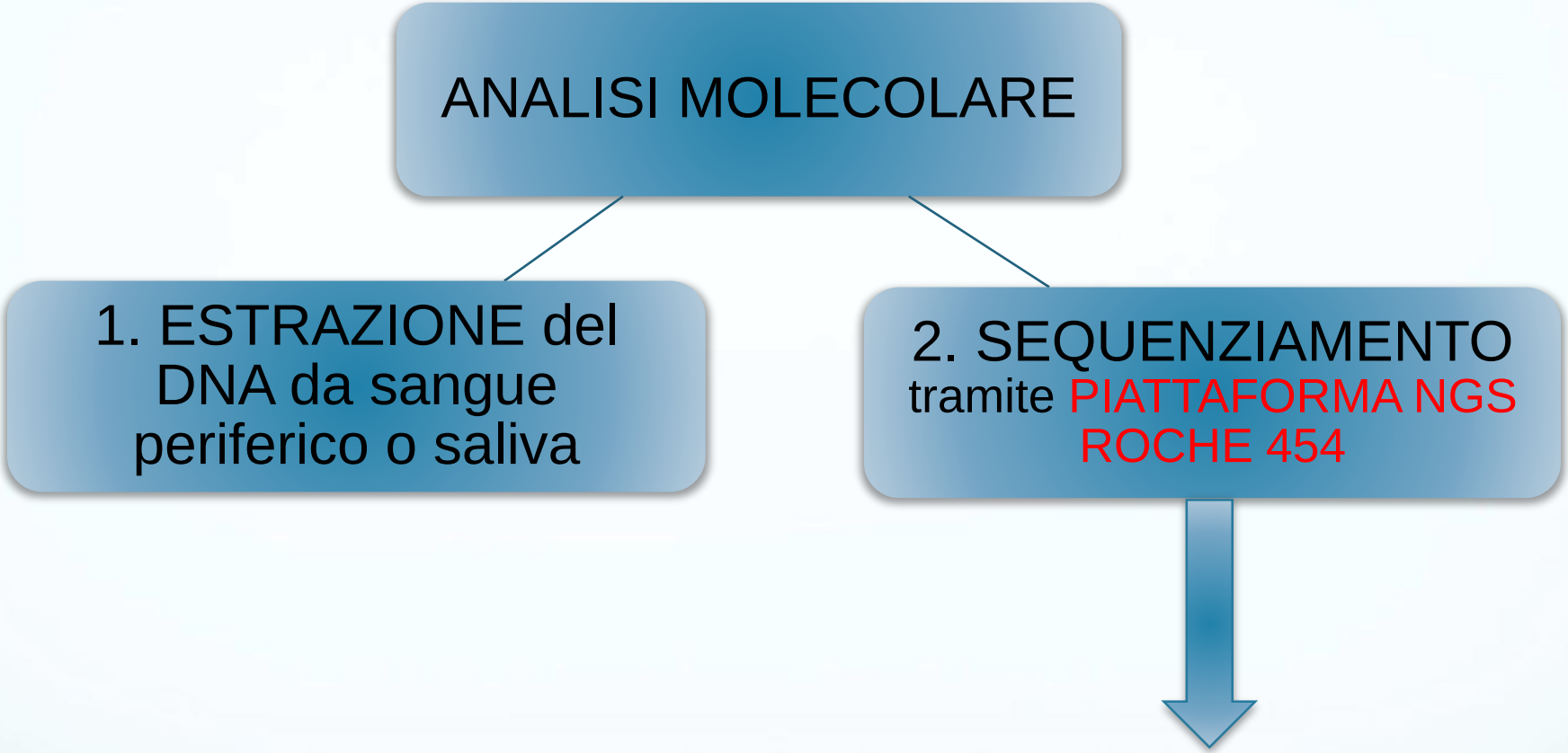
Targeted resequencing in epileptic encephalopathies identifies *de novo* mutations in *CHD2* and *SYNGAP1*



Nature Genetics 2013

ELENCO DEI 36 GENI ANALIZZATI CON NGS

locus	gene	position	locus	gene	position
5q23.2	*ALDH7A1*	chr5:125,877,533-125,931,082	5q14.3	*MEF2C*	chr5:88014058-88179283
Xq11.1-q11.2	*ARHGEF9*	chrX:62854848-62975031	2p16.3	*NRXN1*	chr2:50145643-50574894
Xp21.3	*ARX*	chrX:25021813-25034065	Xq22.1	*PCDH19*	chrX:99546642-99665271
1q23.2	*ATP1A2*	chr1:160085520-160113374	20p12.3	*PLCB1*	chr20:8112912-8865547
19p13.2	*CACNA1A*	chr19:13317256-13617274	19q13.33	*PNKP*	chr19:50364460-50370822
Xp22.13	*CDKL5*	chrX:18525208-18646877	17q21.32	*PNPO*	chr17:46018889-46026674
7q35-q36	*CNTNAP2*	chr7:145813453-148118088	15q26.1	*POLG*	chr15:89859536-89878026
6p12.2	*EFHC1*	chr6:52284994-52360583	2q24.3	*SCN1A*	chr2:166845670-166930149
14q12	*FOXG1*	chr14:29236278-29239483	19q13.12	*SCN1B*	chr19:35521592-35531353
5q34	*GABRG2*	chr5:161494648-161582545	2q24.3	*SCN2A*	chr2:166150341-166248820
16p13.2	*GRIN2A*	chr16:9847265-10276611	2q24.3	*SCN9A*	chr2:167051697-167232497
12p13.1	*GRIN2B*	chr12:13714410-14133022	1q21.3	*SCNM1*	chr1:151138498-151142773
1q23.2	*KCNJ10*	chr1:160007257-160040051	11p15.5	*SLC25A22*	chr11:790475-796263
17q24.3	*KCNJ16*	chr17:68071366-68131746	1p34.2	*SLC2A1*	chr1:43391046-43424847
10q25.3	*KCNK18*	chr10:118957023-118969771	Xq26.3	*SLC9A6*	chrX:135067658-135129286
20q13.33	*KCNQ2*	chr20:62037542-62103993	9q34.11	*SPTAN1*	chrX:135067658-135129286
7q21.11	*MAGI2*	chr7:77646374-79082890	9q34.11	*STXBP1*	chr9:130374486-130454995
Xq28	*MECP2*	chrX:153287264-153363188	15q11.2	*UBE3A*	chr15:25582396-25650653



Metodo del PIROSEQUENZIAMENTO (sequenziamento ad elevato parallelismo)

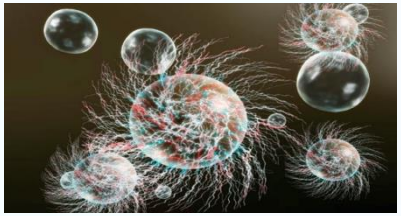
↓

ABBATTIMENTO DEI TEMPI E DEI COSTI DI ANALISI

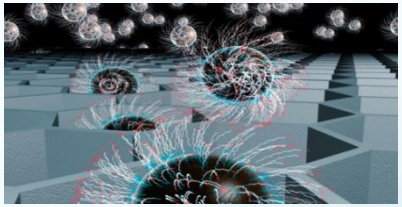
SEQUENZIAMENTO MEDIANTE ROCHE 454

1. PREPARAZIONE DELLE LIBRERIE

2. AMPLIFICAZIONE TRAMITE PCR in emulsione



3.SEQUENZIAMENTO PROPRIAMENTE DETTO



4. ANALISI DEI DATI

ANALISI BIOINFORMATICA

ANNOVAR: IDENTIFICAZIONE SOTTOINSIEME DI VARIANTI FUNZIONALI PATOGENETICHE

Rimozione varianti localizzate in introni e nel 5' e 3' UTR

Eliminazione varianti con frequenza >1% nei database ESP6500-ALL e textit1000g2012feb-ALL

POLYPHEN2, MUTATION TASTER, SIFT, LTR : VALUTAZIONE IN SILICO

Stima del potenziale effetto patogenetico di ogni mutazione

Validazione con Sanger delle varianti con possibile ruolo causativo+ test sui genitori

According to:

1) type of variant (only non **synonymous substitution** are taken into account)

- Missense
- Non sense
- Truncating
- Frameshift

2) In vitro prediction model

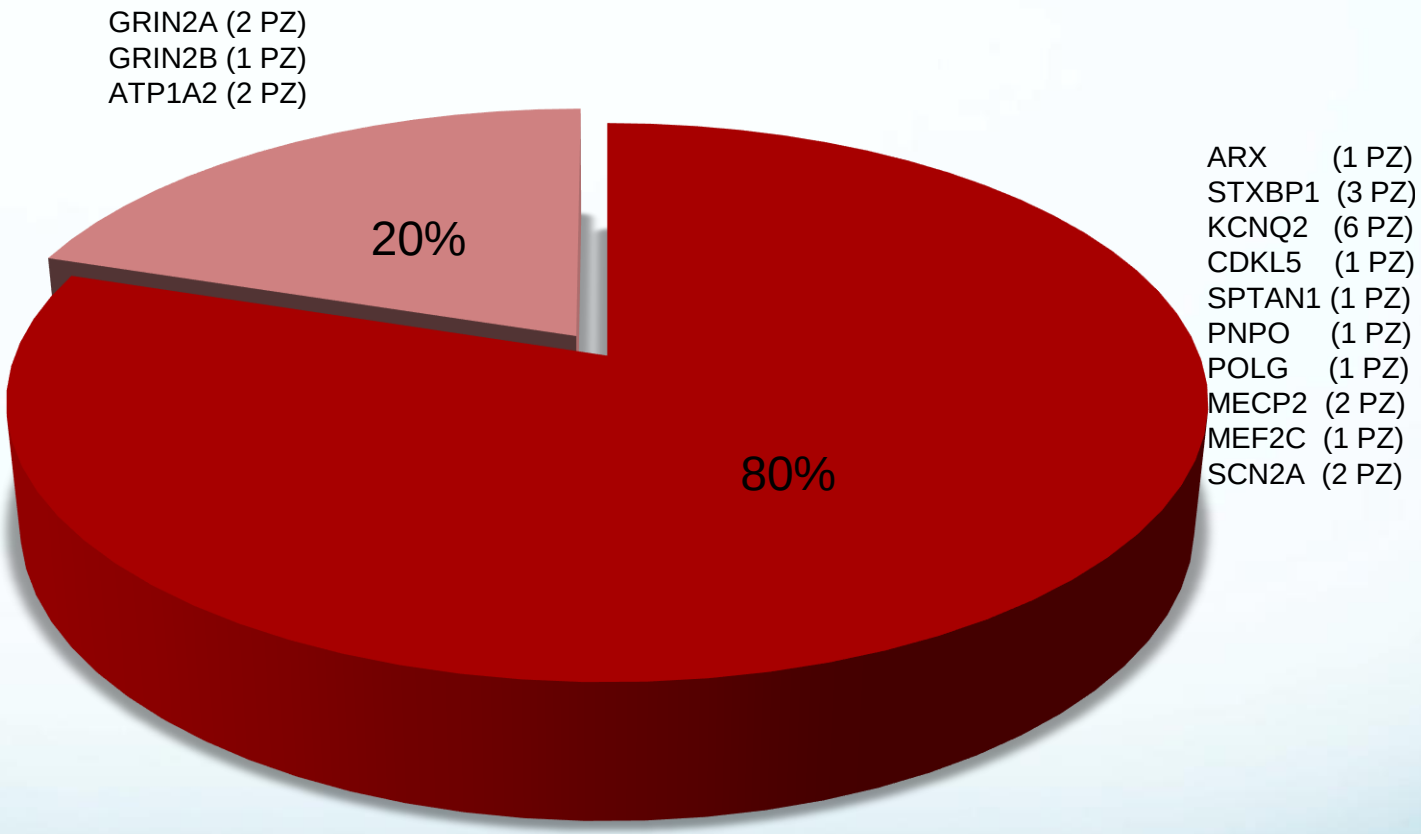
3) inheritance: de novo or inherited

Variants are reported as

- Most likely pathogenic
- Possible pathogenic
- Uncertain
- Unlikely to be pathogenic

RISULTATI

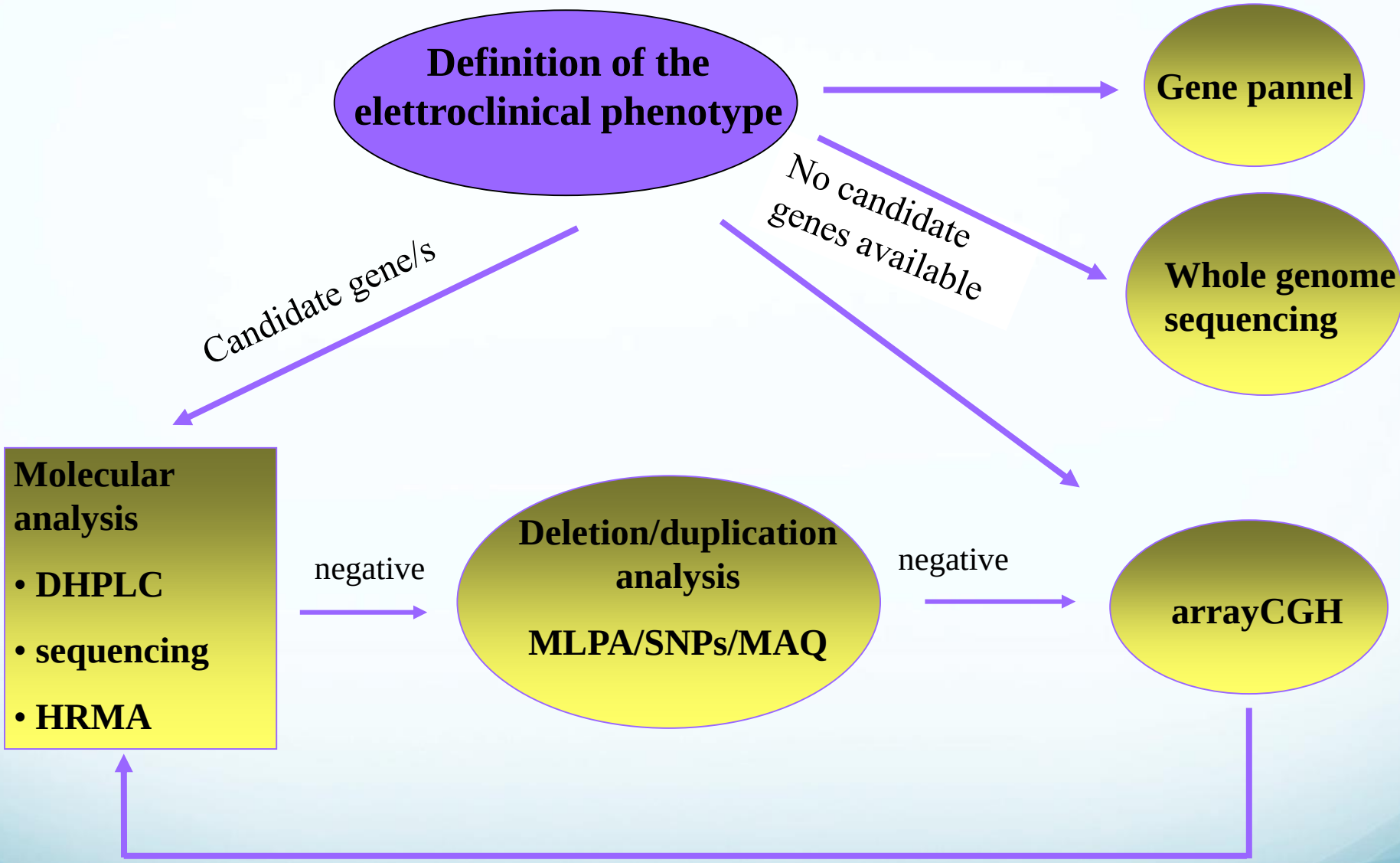
24/127=19%



- mutazioni in GENI con ASSOCIAZIONE NOTA con EOEE
- mutazioni in GENI associati a FENOTIPI ≠

Larger gene panel

- About 100 genes involved in EE including candidate genes emerging from recent WES
- Illumina's sequencing technology
  - sanger validation of interesting variants
  - analysis of variants on the parents
- Advantage: higher number of genes analyzed in a larger cohort of patients, new genotype-phenotype correlation
- Good rapport: quality/time/cost/results
- Problems: bioinformatic analysis of the genes analyzed
  - interpretation of the results
  - genotype-phenotype correlation


Definition of the elettroclinical phenotype
Gene pannel
No candidate genes available
Whole genome sequencing
Candidate gene/s
Molecular analysis
• DHPLC
• sequencing
• HRMA
negative
Deletion/duplication analysis
MLPA/SNPs/MAQ
negative
arrayCGH