Advances on the genetics of Mendelian idiopathic epilepsies

Michel Baulac
Paris Institute of Translational Neurosciences
APHP, Hôpital de la Salpêtrière / Université P&M Curie (Paris VI)
Paris France
Advances on the genetics of Mendelian idiopathic epilepsies

- Idiopathic epilepsies encompasses numerous syndromes as they represent approximately 40% of all epilepsies.

- They are thought to have a genetic origin with a monogenic or polygenic model of inheritance.

- In its "revised terminology and concepts for organization of the epilepsies", the ILAE suggest to include these idiopathic epilepsies in the broader category of genetic syndromes.

- To date, only about 2% of the idiopathic epilepsies are considered to be monogenic.
Since 1995, linkage analysis and “candidate gene” approaches in large families have revealed several genes (KCNQ2, KCNQ3, CHRNA4, CHRNA2, CHRNA2, SCN1B, SCN1A, SCN2A, GABRG2, GABRA1, LGI1) and numerous loci for autosomal dominant febrile seizures and epilepsies.

**Channelopathy concept:** In majority of the monogenic epilepsies the mutated genes encode ion channel subunits, or their receptor, that mediate neuronal excitability and whose gain or loss of function result in abnormal generation and propagation of action potentials.
From Baulac and Baulac, Clinic Neurosc 2010
### Genes and encoded proteins involved in the monogenic epilepsies

<table>
<thead>
<tr>
<th>Protein</th>
<th>Subunit</th>
<th>Gene</th>
<th>Gene locus</th>
<th>Phenotype</th>
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<tr>
<td>Neuronal nicotinic acetylcholinergic</td>
<td>α2-subunit</td>
<td>CHRNA2</td>
<td>8p21</td>
<td>ADNFLE</td>
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<tr>
<td>Neuronal nicotinic acetylcholinergic</td>
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<td>β2-subunit</td>
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<td>M-current protein channel</td>
<td>Kv7.2</td>
<td>KCNQ2</td>
<td>20q13</td>
<td>BFNS</td>
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<tr>
<td>M-current protein channel</td>
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<td>BFNS</td>
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<tr>
<td>Voltage gated Sodium channel</td>
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<td>SCN1A</td>
<td>2q24</td>
<td>GEFS+</td>
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<td>GABA receptor</td>
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<td>GABRA1</td>
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<td>5q34</td>
<td>GEFS+</td>
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<td>Leucine rich glioma inactivated 1</td>
<td></td>
<td>LGI1</td>
<td>10q24</td>
<td>ADPEAF/ADLTE</td>
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</table>
Benign familial neonatal seizures (BFNS)

- Rare, monogenic, autosomal dominant
- Unprovoked, brief cluster of focal tonic–clonic seizures occurring within the first days of life
- Seizures disappear spontaneously within 2 months of life.
- About 10–15% of children with BFNS develop seizures (GTC) later in life; BFNS rarely associated with myokymia, drug-resistant epileptic encephalopathy, and mental retardation
- Mutations KCNQ2 and KCNQ3 (encoding voltage-gated Kv7.2 and Kv7.3 channels). Most KCNQ2 mutations (truncations, deletions) result in haploinsufficiency
- No major phenotypic differences.
- Recent finding of simultaneous microdeletions in KCNQ2 and in CHRNA4, an adjacent gene (ADNFLE). Kurahashi, 2009
Generalized (or Genetic) epilepsy with Febrile Seizures Plus GEFS⁺
GEFS⁺: Generalized (or Genetic) Epilepsy with Febrile Seizures plus

- Febrile seizures (FS)
- Afebrile seizures: mostly GTCs, but also myoclonic, absence, atonic, or partial seizures

Baulac, Lancet Neurology 2004
Main genetic findings in GEFS⁺

- **Locus 19q13**
  - Wallace et al, 1998
  - SCN1B

- **Locus 2q21**
  - Escayg et al, 2000
  - SCN1A

- **Locus 5q31**
  - Baulac et al, 2001
  - Wallace et al, 2001
  - GABRG2

- **Locus 3p24**
  - Audenaert et al, 2005

- **Locus 21q22**
  - Hedera et al, 2006

- **Locus 3q26**

- **Locus 8p23-p21**
  - Baulac et al, 2008

- **Locus 6q16.3-22.31**
  - Poduri et al, 2009

- **Locus 3q26.2-q26.33**
  - Xiao-Hua Dai, 2009
GEFS+: Phenotype/Genotype comparison between SCN1A and GABRG2 mutations

GEFS+ Family with SCN1A mutation

- FS and afebrile TCS [2-8y] Then JME
- Afebrile TCS onset 6y Then JME
- FS [6m-3y] Afebrile TCS onset 3y Then JME
- Several FS TCS (13, 28)
- TCS (adolescence)

- Dravet sd

- SCN1A mutation: M1664K

- Mental Rd Severe ep
- Autism + Epilepsy
FS, GEFS+, Dravet Syndrome spectrum

From Scheffer et al
Severe myoclonic epilepsy in infancy (Dravet syndrome)

- Febrile seizures
- Afebrile seizures
- GTC
- Hemi-clonic
- Myoclonic
- Atypical absence
- Partial
- Status epilepticus

- Motor and Cognitive disorders
- Pharmacoresistance
- High Mortality Rate
Genetic findings in GEFS+, Epileptic encephalopathies with FS

- **Na⁺ channel**

  - Inherited missense mutations: 5-10%
    - FS & epilepsy (GEFS+)

  - De novo mutations: 70-80%
    - Truncating, missense micro-rearrangements
    - Epileptic encephalopathies (Dravet, SMEB, ICE-GTC)

- Familial Benign
- Sporadic cases
  - Pharmacoresistant epilepsy
α1 subunit Na Channel mutations and the continuum between FS, FS+, GEFS+, SMEI (“milder” and classical forms)
Comparison of mis-sense mutations
SMEI (Dravet) vs GEFS+

* Mutation GEFS+
* Mutation SMEI (Dravet)

Kanai, 2009
Parental transmission in SMEI? (Up to 10% of Dravet Syndrome patients)

Mosaic mutations in SCN1A

Sporadic case

Parental transmission

Asymptomatic parents

GEFS+ like phenotype or asymptomatic

Asymptomatic parents

Depienne et al., 2006
Gennaro et al., 2006
Morimoto et al, 2006
Parental mosaïcism in Dravet syndrome

[Genetic analysis diagram and allele-specific PCR results]

Depienne et al, Hum Mut, 2006
Epilepsy and mental retardation limited to females

- Phenotype: from mild to severe in terms of seizure type and severity as well as in the degree of cognitive impairment.
- Seizures begin in infancy or early childhood (6–36 months) and are sensitive to fever in most patients. Variable seizure types including tonic-clonic, tonic, atonic, absences, myoclonic jerks and partial seizures.
- Behavioral problems are often part of the clinical picture and can manifest as autistic, obsessive or aggressive features.

- **PCDH19 gene**, (chromosome Xq22), encodes protocadherin 19, a protein with an unclear biologic role: expressed in the developing brain, role in neuronal connection and signal transduction?
- EFMR affects heterozygous females while hemizygous males are spared.
- Epileptic phenotype may be highly variable between families but also within affected members of the same family.

Scheffer 2008; Dibbens, 2008
Mutations and Deletions in PCDH19 Account for Various Familial or Isolated Epilepsies in Females

• The clinical spectrum associated with PCDH19 mutations can overlap that of Dravet syndrome in females (Depienne, et al., 2009)

• Wide phenotypic expression associated with PCDH19 mutations and deletions
  – from febrile seizures to typical or atypical Dravet, encompassing GEFS+ (Depienne, 2010)

• Reminiscent of what is observed for patients with SCN1A mutations/deletions.
Genetic findings in GEFS+, Epileptic encephalopathies with FS

- **Na\(^+\) channel**
  - Missense mutations
  - De novo mutations: truncating, missense micro-rearrangements
  - 5-10%
  - 70-80%
  - FS & epilepsy (GEFS+)
  - Epileptic encephalopathies (Dravet, SMEB, ICE-GTC)
  - Familial
  - Benign
  - Sporadic cases
  - Pharmacoresistant epilepsy

- **PCDH19**
  - X inactivation?
  - 5-10 %
  - 15%
  - Epilepsy and mental retardation limited to females

*Dibbens 2008, Depienne, 2009*
Family combining childhood absence epilepsy, febrile seizures (FS), and FS+

All affected: locus on 5q: GABRG2 mutation (R43Q)
Childhood absence only: weak linkage on chromosome 13, ...

Marini, 2003
Absence epilepsies with widely variable onset are a key feature of familial GLUT1 deficiency.  

- Familial glucose transporter type 1 (GLUT1) deficiency due to AD inheritance of SLC2A1 mutations is associated with paroxysmal exercise-induced dyskinesia and early-onset absence epilepsy.
- 2 families segregating SLC2A1 mutations identified through probands with early-onset absence epilepsy.
- 15 subjects with SLC2A1 mutations, epilepsy occurred in 12. Absence seizures were the most prevalent seizure type (10/12), with onset from 3 to 34 years of age. Epilepsy phenotypes varied widely, including idiopathic generalized epilepsies (IGE) with absence (8/12), myoclonic-astatic epilepsy (2/12), and focal epilepsy (2/12).
- GLUT1 deficiency is an important monogenic cause of absence epilepsies with onset from early childhood to adult life (not only early-onset absences).
- Individual cases may be phenotypically indistinguishable from common forms of IGE.
- Subtle paroxysmal exertional dyskinesia is a helpful diagnostic clue.
Juvenile myoclonic epilepsy

*Mutations of several genes have been reported in patients with JME*

- Mutations of the EF-hand motif containing 1 (EFHC1) gene has been related to classical JME (encodes a calcium binding protein, which most likely plays a role in calcium homeostasis)
- Anomalies of the GABA$_{A}$ receptor alpha 1 subunit (GABRA1)
- Voltage-gated chloride channel CIC-2 (CLCN2) gene have been discovered in cases of IGE, including JME.

- The involvement of CLCN2 in IGE remains controversial.
- In three unrelated IGE families, 2 novel missense mutations and 1 variant
- Functional characterization of mutant channels predicted a loss of function that may contribute to intracellular chloride accumulation and neuronal hyperexcitability.
- However, incomplete segregation of the mutations among affected members and the transmission by unaffected parents: causative or susceptibility?

*Saint Martin, Hum Mutat.* 2009
Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)

- Brief (5 s–5 min) motor hyperkinetic seizures of frontal origin with tonic or dystonic features presenting in clusters during stage 2 sleep. Daytime seizures may rarely occur.
- Begins in the first 2 decades and is lifelong, but with older age, seizures may become milder and less frequent.
- Mutations in CHRNA4, CHRNB2, or CHRNA2, which encode the neuronal acetylcholine receptor (a4, b2, and a2 subunit, respectively) can be found in around 10–20% of individuals with a positive family history but only in around 5% of individuals with a negative family history.
- The pore-forming M2 transmembrane segments are affected by these mutations, and increased acetylcholine sensitivity is believed to be the main defect of the mutation.
Autosomal Dominant Lateral Temporal epilepsy (ADLTE)

Auditory aura:
- Refrigerator noise
- Radio sound, jingle, song
- Background noises coming to the foreground
- Distortions such as volume change
- Buzzing / ringing / clicking in ears

Secondary generalization in 50%
Precipitation by sounds rarely
Sensory aphasia and visual hallucinations

“I heard voices..”: from semiology, a historical review and a new hypothesis on the presumed epilepsy of Joan of Arc. d’orsi et al, 2006
Autosomal Dominant Lateral Temporal epilepsy (ADLTE)

**General Characteristics**

- ADLTE is a familial temporal lobe epilepsy, characterized by the presence of an auditory aura (ADPEAF)
- Age at onset (# 20yrs, 8-50)
- Not associated with Febrile Seizures, MRI (normal ?)
- From less than 1 per year to monthly attacks, drug-responsive

Mutations in LGI1 have been found in about 50% of ADLTE patients and in about 2% of the non familial patients
Most epilepsy genes encode ion channels, except LGI1

LGI1 gene at 10q23.33; expressed in the brain, neuronal rather than glial predominance.

- LGI1 KO mice display early onset spontaneous seizures & neuronal loss
- Lgi1-mutant rats are epileptic (Baulac S et al, Human Mol Genetics, 2012)
Limbic encephalitis associated with LGI1 antibodies: An autoimmune synaptic encephalopathy

- Memory disorders, confusion/disorientation, seizures (focal, GTC, myoclonic)

Antibodies from patients with limbic encephalitis previously attributed to voltage-gated potassium channels recognise LGI1, a neuronal secreted protein that interacts with presynaptic ADAM23 and postsynaptic ADAM22.

Lai, 2010
Lgi1 functions

- Role in the **maturation of glutamatergic synapses** during postnatal development

  [Images of WT Lgi1 and Truncated Lgi1]

  Zhou et al., Nat Med 2009

- Role in the **glutamatergic transmission**

  ADAM 22, a transmembrane protein involved in the development, activity, and plasticity of excitatory synapses

  Modulator of postsynaptic AMPA R clustering?

  LGI1 forms also complexes with Voltage-gated Potassium channels VGKC

  Fukata et al., Science 2006
Lgi1 KO mice: Increased excitatory transmission in CA1 pyramidal cells

Loss of Lgi1 cause glutamatergic transmission potentiation (without affecting inhibitory transmission)
Advances on the genetics of Mendelian idiopathic epilepsies: Conclusions

• Rare, but they permitted the discovery of major epilepsy genes and to develop the concept of channelopathies: AchR, GABA$_A$R, Na$^+$ channel, K$^+$ channel, Cl$^-$ channel

Goals of basic and clinical research include
• Finding new genes: new generation of sequencing technologies (plus insertions, deletions, etc..)
• Improving phenotype/genotype correlations
• Approaching mechanisms of epileptogenesis and ictogenesis, and identifying thereby new drug targets
Team ICM

Permanent Researchers
Stéphanie Baulac, PhD
Christel Depienne, PhD
Eric LeGuern, MD, PhD
PhD student
Morgane Boillot
Master student
Dabhis Agher
Julie Renard
Technician
Eric Noé

Epilepsy Unit
(Michel Baulac, Vincent Navarro and coll)

National Centre for rare epilepsies
Isabelle An, MD, PhD
Rima Nabbout, MD, PhD
Olivier Dulac, MD, PhD

Neurogenetics Lab
Cécile Cazeneuve, PharmD, PhD
Christel Depienne, PhD
Eric LeGuern, MD, PhD
Benign familial neonatal/infantile seizures, and benign familial infantile seizures

- BFNS, BFNIS, and BFIS: classified on the basis of age at onset
- BFNIS: neonates; BFIS: from the 3rd and the 12th month of life.
- Seizures usually focal, with or without secondary generalisation.
- Mutations in the voltage-gated sodium channel alpha 2 subunit (SCN2A) have been reported in BFNIS and BFIS (Gain of function)

- Benign infantile seizures may also be associated with paroxysmal dyskinesia, a movement disorder in the form of choreoathetosis or dystonia, generating the infantile convulsions with choreoathetosis (ICCA) syndrome (AD, but ICCA gene not mapped).
Two novel CLCN2 mutations accelerating chloride channel deactivation are associated with idiopathic generalized epilepsy

- Heterozygous mutations in the CLCN2 gene encoding the voltage-gated chloride channel CLC2 have been identified in patients with IGE
- The involvement of CLCN2 in epilepsy (IGE) remains controversial.

- In three unrelated IGE families, 2 novel missense mutations, p.Arg235Gln and p.Arg577Gln, (absent in large ethnically-matched control populations), and one novel p.Arg644Cys variant, (also found in five controls).
- Functional characterization of mutant channels predicted a loss of function that may contribute to intracellular chloride accumulation or neuronal hyperexcitability.
- However, the incomplete segregation of the mutations among affected members and the transmission by unaffected parents suggested that these CLCN2 mutations alone are not sufficient to induce epilepsy.
- They may instead represent susceptibility factors among other so far undetected genetic alterations in the respective families

*Saint Martin, Hum Mutat.* 2009
Juvenile myoclonic epilepsy

Mutations of several genes have been reported in patients with JME

- Mutations of the EF-hand motif containing 1 (EFHC1) gene has been related to classical JME (encodes a calcium binding protein, which most likely plays a role in calcium homeostasis)
- Anomalies of the GABA\(_A\) receptor alpha 1 subunit (GABRA1)
- Voltage-gated chloride channel CIC-2 (CLCN2) genes have been discovered in cases of IGE, including JME.

- Higher frequency of CHRNA4 1674(+11)C>T polymorphism has been observed in JME patients, suggesting that CHRNA4 may be one of the candidate genes
- The EFHC1 gene.
- GABA\(_A\) receptors are ligand-gated chloride channels, which carry out inhibitory functions
- Chloride currents are also altered in case of CLCN2 gene mutations, since anomalies of the CIC-2 channels determine the impairment of chloride efflux, with intracellular accumulation of chloride
Table 1. Genetic Loci and Known Genes Associated with Dravet Syndrome, GEFS+, and Febrile Seizures

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene</th>
<th>Dravet</th>
<th>GEFS+</th>
<th>FS</th>
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</thead>
<tbody>
<tr>
<td>19q13</td>
<td>SCN1B</td>
<td>+</td>
<td>+ (GEFS+1)</td>
<td>+</td>
</tr>
<tr>
<td>2q24</td>
<td>SCN1A</td>
<td>+ (high frequency)</td>
<td>+ (GEFS+2)</td>
<td>+</td>
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<td>5q31.1-q33.1</td>
<td>GABRG2</td>
<td>+</td>
<td>+ (GEFS+3)</td>
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<td>2q24</td>
<td>SCN2A</td>
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<tr>
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<td>SCN9A</td>
<td>+</td>
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<tr>
<td>2q22-q23</td>
<td>CACNB4</td>
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<tr>
<td>Xq22</td>
<td>PCDH19</td>
<td>+ (females)*</td>
<td>+ (females)</td>
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</tr>
</tbody>
</table>

* Dravet-like features predominantly associated with febrile seizures (Depienne et al 2011).
Autosomal Dominant Nocturnal Frontal Lobe Epilepsy.

Authors
- Hirose S, Kurahashi H.

Editors

Source
- GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-. 2002 May 16 [updated 2010 Apr 05].

Excerpt

DISEASE CHARACTERISTICS:
- Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is characterized by clusters of nocturnal motor seizures, which are often stereotyped and brief (5 seconds to 5 minutes). They vary from simple arousals from sleep to dramatic, often bizarre, hyperkinetic events with tonic or dystonic features. Affected individuals may experience aura. Retained awareness during seizures is common. A minority of individuals experience daytime seizures. Onset ranges from infancy to adulthood. About 80% of individuals develop ADNFLE in the first two decades of life; mean age of onset is ten years. Neurologic examination is normal and intellect is usually preserved. Within a family, the manifestations of the disorder may vary considerably. ADNFLE is lifelong but not progressive. As an individual reaches middle age, attacks may become milder and less frequent.

DIAGNOSIS/TESTING:
- The diagnosis of ADNFLE is made on clinical grounds. A detailed history from the affected individual and witnesses, supplemented if necessary by video-EEG monitoring, is the key to diagnosis. Clinically available molecular genetic testing reveals mutations in CHRNA4, CHRNB2, or CHRNA2 in approximately 10%-20% of individuals with a positive family history and fewer than 5% of individuals with a negative family history.

MANAGEMENT:
- Treatment of manifestations: Carbamazepine is associated with remission in about 70% of individuals, often in relatively low doses. Individuals with ADNFLE associated with the CHRNA4 mutation p.Ser284Leu are more responsive to zonisamide than carbamazepine. Resistance to AEDs, present in about 30% of affected individuals, requires a trial of all appropriate AEDs. Testing of relatives at risk: A medical history from relatives at risk can identify those with ADNFLE so that treatment can be initiated promptly.

GENETIC COUNSELING:
- ADNFLE is inherited in an autosomal dominant manner. Most individuals diagnosed with ADNFLE have an affected parent. The proportion of cases caused by de novo gene mutations is unknown, as the frequency of subtle signs of the disorder in parents has not been thoroughly evaluated and molecular genetic data are insufficient. Penetration is estimated at 70% and the risk to each offspring of inheriting the mutant allele is 50%; thus, the chance that the offspring will manifest ADNFLE is (50%)(70%)=35%. Prenatal testing for pregnancies at increased risk may be available through laboratories offering custom prenatal testing if the disease-causing mutation in
Familial form of typical childhood absence epilepsy in a consanguineous context.


Source
Service de Neurologie, CHU Razi, La Manouba, Tunisie.

Abstract
Causative genes for childhood absence epilepsy (CAE) are unknown partly because families are small or phenotypically heterogeneous. In five consanguineous Tunisian families with at least two sibs with CAE, 14 patients fulfilled the diagnostic criteria for CAE (Epilepsia 1989; 30:389-399). Linkage analyses or direct sequencing excluded CACNG2, CACNA1A, CACNB4, and CACNA2D2, orthologs of genes responsible for autosomal recessive (AR) absence seizures in mice. These families will help identify (a) gene(s) responsible for CAE.
Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome.


Source
INSERM U975 (CRicm), Bâtiment Pharmacie 4 étage, Groupe Hospitalier Pitié-Salpêtrière, 47 boulevard de l'hôpital, 75013 Paris, France. christel.depienne@upmc.fr

Abstract
BACKGROUND Mutations in SCN1A can cause genetic epilepsy with febrile seizures plus (GEFS+, inherited missense mutations) or Dravet syndrome (DS, de novo mutations of all types). Although the mutational spectra are distinct, these disorders share major features and 10% of DS patients have an inherited SCN1A mutation. OBJECTIVES AND PATIENTS 19 selected families with at least one DS patient were studied to describe the mechanisms accounting for inherited SCN1A mutations in DS. The mutation identified in the DS probands was searched in available parents and relatives and quantified in the blood cells of the transmitting parent using quantitative allele specific assays. RESULTS Mosaicism in the blood cells of the transmitting parent was demonstrated in 12 cases and suspected in another case. The proportion of mutated allele in the blood varied from 0.04-85%. In the six remaining families, six novel missense mutations were associated with autosomal dominant variable GEFS+ phenotypes including DS as the more severe clinical picture. CONCLUSION The results indicate that mosaicism is found in at least 7% of families with DS. In the remaining cases (6/19, 32%), the patients were part of multiplex GEFS+ families and seemed to represent the extreme end of the GEFS+ clinical spectrum. In this latter case, additional genetic or environmental factors likely modulate the severity of the expression of the mutation.
The genetics of Dravet syndrome

- 70% -80% : sodium channel α1 subunit gene (SCN1A) abnormalities:
  - Truncating mutations account for about 40% and have a significant correlation with an earlier age of seizures onset.
  - The remaining SCN1A mutations comprise splice-site and missense mutations, most of which fall into the pore-forming region of the sodium channel. Mutations randomly distributed across SCN1A protein.
  - Most mutations are de novo, but familial SCN1A mutations also occur. Somatic mosaic mutations have been reported in some patients and might explain the phenotypical variability seen in some familial cases.
  - SCN1A exons deletions or chromosomical rearrangements involving SCN1A and contiguous genes are also detectable in about 2-3% of patients.
- A small percentage of female patients with a DS-like phenotype might carry PCDH19 mutations.
- Rare mutations have been identified in the GABARG2 and SCN1B genes.
- The etiology of about 20% of DS patients remains unknown, and additional genes are likely to be implicated.

INTRODUCTION:
Mutations in the voltage-gated sodium channel SCN1A gene are the main genetic cause of Dravet syndrome (previously called severe myoclonic epilepsy of infancy or SMEI).

OBJECTIVE:
To characterise in more detail the mutation spectrum associated with Dravet syndrome.

METHODS:
A large series of 333 patients was screened using both direct sequencing and multiplex ligation-dependent probe amplification (MLPA). Non-coding regions of the gene that are usually not investigated were also screened.

RESULTS:
SCN1A point mutations were identified in 228 patients, 161 of which had not been previously reported. Missense mutations, either (1) altering a highly conserved amino acid of the protein, (2) transforming this conserved residue into a chemically dissimilar amino acid and/or (3) belonging to ion-transport sequences, were the most common mutation type. MLPA analysis of the 105 patients without point mutation detected a heterozygous microrearrangement of SCN1A in 14 additional patients; 8 were private, partial deletions and six corresponded to whole gene deletions, 0.15-2.9 Mb in size, deleting nearby genes. Finally, mutations in exon 5N and in untranslated regions of the SCN1A gene that were conserved during evolution were excluded in the remaining negative patients.

CONCLUSION:
These findings widely expand the SCN1A mutation spectrum identified and highlight the importance of screening the coding regions with both direct sequencing and a quantitative method. This mutation spectrum, including whole gene deletions, argues in favour of haploinsufficiency as the main mechanism responsible for Dravet syndrome.
OBJECTIVE:
Two unrelated families were ascertained in which sisters had infantile onset of epilepsy and developmental delay. Mutations in the protocadherin 19 (PCDH19) gene cause epilepsy and mental retardation limited to females (EFMR). Despite both sister pairs having a PCDH19 mutation, neither parent in each family was a heterozygous carrier of the mutation. The possibility of parental mosaicism of PCDH19 mutations was investigated.

METHODS:
Genomic DNA from peripheral blood was obtained and sequenced for PCDH19 mutations. Parentage was confirmed by markers.

RESULTS:
Both sister pairs have a mutation in PCDH19. Sister pair 1 has a missense mutation, c.74T>C, L25P, while sequence analysis indicates both of their parents are negative for the mutation. Diagnostic restriction enzyme analysis detected low-level mosaicism of the mutation in their mother. Sister pair 2 are half-sisters who share a mother and each has the missense PCDH19 mutation c.1019 A>G, N340S. The sequence chromatograph of their mother shows reduced signal for the same mutation. These data indicate maternal somatic and gonadal mosaicism of the PCDH19 mutation in both sister pairs. Phenotyping is suggestive of, and PCDH19 mutation detection is diagnostic for, the disorder EFMR in the affected girls.

CONCLUSIONS:
We show that gonadal mosaicism of a PCDH19 mutation in a parent is an important molecular mechanism associated with the inheritance of EFMR. This should be considered when providing genetic counseling for couples who have one affected daughter as they may risk recurrence of affected daughters and having sons at risk of transmitting EFMR.
Epilepsy and mental retardation limited to females with PCDH19 mutations can present de novo or in single generation families.


Source
SA Pathology, Women's and Children's Hospital, 72 King William Road, North Adelaide, SA 5006, Australia.

Abstract

BACKGROUND:
Epilepsy and mental retardation limited to females (EFMR) is an intriguing X-linked disorder affecting heterozygous females and sparing hemizygous males. Mutations in the protocadherin19 (PCDH19) gene have been identified in seven unrelated families with EFMR.

METHODS AND RESULTS:
Here, we assessed the frequency of PCDH19 mutations in individuals with clinical features which overlap those of EFMR. We analysed 185 females from three cohorts: 42 with Rett syndrome who were negative for MECP2 and CDKL5 mutations, 57 with autism spectrum disorders, and 86 with epilepsy with or without intellectual disability. No mutations were identified in the Rettsyndrome and autism spectrum disorders cohorts suggesting that despite sharing similar clinical characteristics with EFMR, PCDH19 mutations are not generally associated with these disorders. Among the 86 females with epilepsy (of whom 51 had seizure onset before 3 years), with or without intellectual disability, we identified two (2.3%) missense changes. One (c.1671C-->G, p.N557K), reported previously without clinical data, was found in two affected sisters, the first EFMR family without a multigenerational family history of affected females. The second, reported here, is a novel de novo missense change identified in a sporadic female. The change, p.S276P, is predicted to result in functional disturbance of PCDH19 as it affects a highly conserved residue adjacent to the adhesion interface of EC3 of PCDH19.

CONCLUSIONS:
This de novo PCDH19 mutation in a sporadic female highlights that mutational analysis should be considered in isolated instances of girls with infantile onset seizures and developmental delay, in addition to those with the characteristic family history of EFMR.
CA1 pyramidal cells recordings: increased excitatory transmission

AMPA currents

P8 = before seizures onset: increased mEPSC amplitude

mEPSC = miniature excitatory postsynaptic currents

Baulac S, Unpublished data, Coll. Nathalie Rouach, Collège de France
Retigabine-A Unique Mechanism of Action: A Neuronal Potassium-Channel Opener

Ribbon schematic of KCNQ2 channel subunit 5th (S5) and 6th (S6) transmembrane domains and P-loop (P), comprising the selectivity filter, pore, and gate. Retigabine binds near the gate, favoring channel opening and the outward flow of potassium ions.
GEFS$^+$ families unrelated to SCN1A, SCN1B, GABRG2 mutations

- Mutations have been identified in 10–15% of autosomal dominant GEFS$^+$ families.

- 7 families, 167 individuals (Bonani 2004)
  - 41 had epilepsy, 29 a phenotype consistent with GEFS+, 7 IGE, 3 unclassifiable, 2 phenocopies
  - Phenotypes included FS$^+$ (29%), FS (29%), IGE (18%), FS$^+$ and focal seizures (13%) or absences (3%) and FS with absences (2%)
Families combining FS and Temporal Lobe seizures (GEFS+?)

• TLE in GEFS+ as a sequela of prolonged FS
  – Family with FS, GTC seizures and partial epilepsy, including TLE: *SCN1A mutation* (Abou-khalil 2001); Singh 1999

• TLE as a GEFS+ phenotype, not simply as a secondary effect of damage from GTC seizures
  – Families combining FS and TLE without MRI-detectable hippocampal sclerosis (Baulac 2001, Depondt 2002)
  – Missense mutation (M145T) SCN1A: 13 with simple FS, 3 with TLE (2 with Hippocampal sclerosis) (Colossimo 2007)
  – GEFS+ families with SCN1B mutations (Scheffer, 2007)
GEFS$^+$ families versus Autosomal Dominant Febrile Seizure families

- High proportion of FS and FS$^+$ in GEFS$^+$ families

- Autosomal Dominant FS families suggested loci for FS
  - $FEB1$ 8q13–q21 Australia Wallace et al. (1996)
  - $FEB2$ 19p13.3 USA Johnson et al. (1998)
  - $FEB3$ 2q23–q24 USA Peiffer et al. (1999)
  - $FEB4$ 5q14–q15 Japan Nakayama et al. (2000)
  - $FEB5$ 6q22–q24 France Nabbout et al. (2002)
  - $FEB6$ 18p11.2 Japan Nakayama et al. (2004)
GEFS\(^+\) families versus Autosomal Dominant Febrile Seizure families

- But most of these ADFS families also include individuals with afebrile seizures
- \textit{FEB3} is the same locus as for some SCN1A-linked GEFS\(^+\) families; GABRG2 was found in association studies of individuals with FS
- Families meeting the requirements for both ADFS and the broader definition of GEFS\(^+\) (Hindocha, 2008)

- GEFS\(^+\) and ADFS have a clinical (and genotypic?) overlap.
New gene involved in Dravet and related conditions

- 27% of Dravet S patients negative for mutations or rearrangements in SCN1A (as well as for other known mutations)
- PCDH19, a new gene on chromosome X
  - recently found in a familial epileptic syndrome known as female-limited epilepsy and cognitive impairment.
  - Clinical picture compatible with Dravet Syndrome: association of early febrile and afebrile seizures, seizures occurring in clusters, developmental and language delays, behavioural disturbances, and cognitive regression. Slight but constant differences in the evolution of the patients, including fewer polymorphic seizures (in particular rare myoclonic jerks and atypical absences)
- Protocadherin19: transmembrane protein involved in establishment of neuronal connections during development
Idiopathic epilepsies

Focal
- FPEVF & FMLTE
- ADNFLE
- ADLTLE

Generalized
- SMEI & Borderline
- GEFS+
- JME

Indetermined
- BFNIS
- BFNS
- BFIS
Lgi1 a new protein involved in epilepsy and more...

- Most epilepsy genes encode ion channels, except LGI1

LGI1 is mutated in 50% of familial temporal lobe epilepsies (ADLTLE) with auditory features

- *Lgi1* -/- mice have spontaneous seizures (Chabrol et al, Brain 2010)
- *Lgi1* +/- mice are susceptible to audiogenic seizures
Most monogenic idiopathic epilepsies belong to the neuronal channelopathies.
Using large families with AD inheritance, great progress have been obtained in the identification of new epilepsy genes and thus the understanding of molecular pathways.

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Autosomal Dominant Lateral Temporal epilepsy (ADLTE)

Mutations in LGI1 have been found in about 50% of the ADLTE patients and in about 2% of the non-familial patients.
Na+ neuronal channelopathy (SCN1A) SMEI (Dravet) vs GEFS+ 

Missense inherited mutations \( \rightarrow \sim 10\% \)

de novo nonsense mutations (mostly) + deletions \( \rightarrow \sim 70\% \)

GEFS+ 
Familial Benign

Epileptic encephalopathy (Dravet, SMEB, ICE-GTC) 
Sporadic Pharmaco-resistant & severe

Marini C, 2007; Lossin, 2008
Benign familial neonatal seizures BFNS

• Rare, monogenic, autosomal dominant
• Unprovoked, brief cluster of focal tonic–clonic seizures occurring within the first days of life and frequently flowing into status epilepticus. (+ Apnoeic and GTCs fits)
• Individuals can be kept seizure-free using phenobarbital.
• Seizures disappear spontaneously within 2 months of life.
• About 10–15% of children with BFNS develop seizures (GTC) later in life, with a variable age of onset and duration; EEG may be characterized by centrotemporal spikes and sharp waves
• BFNS rarely associated with peripheral nerve hyperexcitability (myokymia), therapy-resistant epileptic encephalopathy, and variable degree of mental retardation
BFNS: mutations in the KCNQ2 and KCNQ3 genes, members of a family of voltage-gated potassium channel genes (KCNQ1–5).

- KCNQ2 and KCNQ3 predominantly expressed in the brain, mainly hippocampus, temporal cortex, cerebellum, and medulla oblongata, from late foetal life to early infancy, coinciding with the time in which BFNS occurs.
- They encode the voltage-gated Kv7.2 and Kv7.3 channels that produce a muscarinic-regulated potassium current (M-current), a slow potassium current important in the modulation of the resting membrane potential. This action limits the repetitive firing of many neurons.
- In majority KCNQ2. 50% of KCNQ2 mutations are truncations, splice-site defects, or deletions or insertions; about 60% of these mutations are in the C-terminus and are predicted to cause truncation of the C-terminus with haploinsufficiency. Missense mutations have been reported in KCNQ3.
- No major phenotypic differences KCNQ2 and KCNQ3 mutation.
- Small number BFNS families, genotype–phenotype correlations speculative.
- Penetrance incomplete (85%); anticipation not observed. Majority of newborns with BFNS have an affected parent; however, sporadic BFNS has also been reported.