**KCNQ2 gene discovery; Epilepsy mechanism, and other ion channel mutations in epilepsy**

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Financial Disclosures

- No relevant financial relationships with any commercial interests.
INTRODUCTION: WHAT IS EPILEPSY?
Epilepsy

Common
- Prevalence 4-8/1000
- Life time incidence 3%

Key symptom = seizures
- Abnormal synchronization of cortical neurons
- Hyperexcitable neurons

Epilepsy = channelopathy
Action potential

Repolarization
(closure of Na⁺ and opening of K⁺, voltage gated channels)

Depolarization
(opening of voltage gated Na⁺ channels)

(local potential change: graded potential)

Hyperpolarization
(Voltage gated K⁺ channels remain open after the potential reaches resting level)

Threshold level

RMP

Time (ms)

+35

0

mv

−65

−90

(1 - 4 ms)
Ligand and voltage gated ion channels

H. Lerche et al., J. Physiol. 2013
Epilepsy

↓ Inhibition

↑ Excitation
Causes of epilepsy

Figure 1 Advances in understanding the causes of epilepsy

Thomas, R. H. & Berkovic, S. F. (2014) The hidden genetics of epilepsy—a clinically important new paradigm

Nat. Rev. Neurol. doi:10.1038/nrneurol.2014.62
### Monogenetic epilepsies (non-exhaustive)

<table>
<thead>
<tr>
<th>Ion channel genes</th>
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<tr>
<td>GABRB3</td>
<td>PCDH19</td>
<td>TBC1D24</td>
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Ion channel genes
THE GENE KCNQ2 IN EPILEPSY
Voltage gated potassium channels

- Humans: > 70 potassium channel genes

- Small family of voltage gated KCNQ genes: KCNQ1-5
  - KCNQ1: cardiac arrhythmia (LQTS)
  - KCNQ2/3: epilepsy
  - KCNQ4: deafness
KCNQ2

• Encoding voltage gated potassium channel subunit K\textsubscript{\text{v}}7.2
  • Hyperpolarizing M current
  • Stabilizes neuronal excitability
Heteromeric \textit{KCNQ2/KCNQ3} channels

Front. Pharmacol., 23 March 2012
Benign Familial Neonatal Seizures (BFNS)

- Autosomal dominant
- Seizures onset between 2 - 8 days, remission within first months of life
- Seizures: tonic -> autonomic and motor changes, uni- or bilateral. Often with episodes of apnea
- Investigations normal
- Psychomotor development normal
- Small increased risk of recurring seizures later in life
Missense, nonsense, splicing, frameshift mutations, intragenic insertions/deletions, whole gene deletions described
Where the story starts

- **KCNQ2** screening offered for neonatal seizures in diagnostic unit
  - 2010: 1 patient: refractory seizures and psychomotor regression

- Literature
  - 4 case reports of patients with neonatal seizures and intellectual disability
Methods

• KCNQ2 and KCNQ3 screening in 80 patients with unexplained neonatal or early onset epileptic encephalopathy
  • Onset < 3 months
  • Slowing of psychomotor development
  • Metabolic screening normal
  • Imaging: no explanation
  • Genetic screening for relevant genes normal

Results

- No KCNQ3 mutations
- 7 novel KCNQ2 missense mutations in 8/80 patients (10%)
- Not present in 276 ethnically matched controls
- Inheritance
  - 6 mutations de novo
  - 1 paternal DNA unavailable
  - 1 mosaic father

KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy.


Neurogenetics Group, VIB-Department of Molecular Genetics, University of Antwerp, Antwerp, Belgium.
Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation.


Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2.


Extending the KCNQ2 encephalopathy spectrum: Clinical and neuroimaging findings in 17 patients.


KCNQ2 encephalopathy: delineation of the electroclinical phenotype and treatment response.

• **September 2014:**
  - 62 patients with KCNQ2 encephalopathy described in literature
    - 44 different mutations
  - 23 additional non-reported European patients
    - 8 novel mutations
**KCNQ2 encephalopathy**

- 10% of patients with neonatal EE of unknown etiology
- **KCNQ2 encephalopathy mutations**
  - All missense mutations
    - Several mutations recurrent in multiple patients
    - Several mutations in same codon
  - Novel: not reported in BFNS
- **Inheritance**
  - *de novo*
  - 1 mosaic father with BNS phenotype
KCNQ2 encephalopathy

- **Neonatal onset**
  - 1 patient onset at 5 months
- **Seizure type at onset**
  - Prominent tonic component
  - Often autonomic features: apnea, desaturation, bradycardia
- **Dramatic onset, multiple sz daily**
- **EEG at onset** burst-suppression pattern or multifocal epileptic activity
- **Range of cognitive outcome, mostly severe to profound intellectual disability**
MECHANISM
BFNS → \textit{KCNQ2} mutation → encephalopathy

20-50\% reduction of M current.
(Few cases with dominant negative effect: BFNS+myokymia)
=> Haploinsufficiency
Mechanism

Orhan et al., Annals of Neurology, 2014
5/7 mutations: dominant negative effect on channel function

Orhan et al., Annals of Neurology, 2014
Mouse model of dominant negative mutation

- Transgenic adult mice carrying dominant negative KCNQ2 mutation
  - recurrent spontaneous seizures
  - impaired spatial learning
  - marked hyperactivity
- Pyramidal neurons
  - diminished M-type K+ current, hyperexcitable
- Studies of hippocampal sections
  - laminar disorganization of area CA1
KCNQ2 spectrum

Functional aspects mutation + genetic background

BFNS  KCNQ2  KCNQ2 encephalopathy

- Mostly Inherited
- Mostly de novo
- Mozaicism described in inherited cases
SCN1A

- Encoding Na\textsubscript{v} 1.1
1997
Clinical description GEFS+ syndrome (Scheffer et al.)

2000
SCN1A mutation in large GEFS+ family (Escayg et al.)

2001
SCN1A mutation in 7/7 patients with Dravet syndrome (Claes et al.)

Follow up studies: SCN1A mutations/deletions in 70-80% of Dravet patients
**Action potential**

**Depolarization**
(Opening of voltage gated Na⁺ channels)

**Repolarization**
(Closure of Na⁺ and opening of K⁺, voltage gated channels)

**Hyperpolarization**
(Voltage gated K⁺ channels remain open after the potential reaches resting level)

**Threshold level**

**RMP**

**mv**

**Time (ms)**

-35

-65

-90

(1-4 ms)
LOF mutation in excitatory ion channel subunit

??

Epilepsy
GEFS+  SCN1A mutation  Dravet
**General Rule**

- **GEFS+**
  - Missense mutations
    - Outside pore region

- **SMEI**
  - Truncating mutations, splice site mutations, deletions
  - Missense mutations
    - In pore region
    - More often changes in AA polarity

BUT exceptions!!
Functional aspects mutation + genetic background

BFNS  **KCNQ2**  KCNQ2 encephalopathy
- Mostly Inherited
- Mostly *de novo*
- Mozaicism described in inherited cases

GEFS+  **SCN1A**  Dravet Syndrome

Functional aspects mutation + genetic background
SCN2A
- Encoding \( \text{Na}_v 1.2 \)
SCN2A in BFNIS

- Benign familial neonatal-infantile seizures
  - Autosomal dominant
  - Seizures onset between 2 days and 7 months, remission by 12 months of life
  - Investigations normal; Psychomotor development normal

2002: SCN2A mutations in BFNIS. All missense mutations
SCN2A encephalopathy

De novo SCN2A splice site mutation in a boy with Autism spectrum disorder.

Shi X, Yasumoto S, Nakagawa T.

De novo mutations of voltage-gated sodium channel gene SCN2A in intractable epilepsies.


Whole genome sequencing identifies SCN2A mutation in monozygotic twin with Dravet syndrome and unique neuropathologic findings.


Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome.

Genotype-phenotype correlation

• Across all phenotypes
  • Most mutations = missense mutations
    • BFNIS predominantly in transmembrane domains (TMD), “severe” mutations more outside TMD
    • Both net gain vs. loss of function described
  • All truncating mutations => EE/ID/autism

• No conclusive results (yet)
SCN2A spectrum

Functional aspects mutation + genetic background

BFNS  KCNQ2  KCNQ2 encephalopathy

BFNIS  SCN2A  SCN2A encephalopathy

GEFS+  SCN1A  Dravet Syndrome

Functional aspects mutation + genetic background
**KCNT1**

- Sodium-gated potassium channel
- Slow hyperpolarization that follows repetitive firing
- C-terminal cytoplasmic domain interacts with protein network including FMRP
Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy.

De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy.
### ADNFLE

Heron et al., 2012

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of affected individuals</th>
<th>Origin</th>
<th>Age of onset</th>
<th>Other clinical features</th>
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<td></td>
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<td>Mean ± s.d.</td>
<td>Median (range) in years</td>
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<td>A</td>
<td>6</td>
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<td>4.6 ± 5.9</td>
<td>2 (1–15)	extsuperscript{d}</td>
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<tr>
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<td>5.5 (3–8)</td>
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<tr>
<td>C</td>
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<td>Israeli (Sephardic Jewish)</td>
<td>8.5 ± 6.4</td>
<td>5.5 (5–18)</td>
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<td>D</td>
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<td>Australian (British descent)</td>
<td>9.0</td>
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MMPSI (EIMFS)

- Onset < 6 months
- Polymorphic focal seizures
- Migrating ictal EEG pattern
- Arrest of psychomotor development

<table>
<thead>
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<td>p.Arg474His</td>
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<td>c.2280C&gt;G</td>
<td>p.Ile760Met</td>
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Barcia et al., 2012
Milligan et al., Annals of Neurology 2014
<table>
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<tr>
<th>Gene</th>
<th>Benign</th>
<th>Severe/Epileptic encephalopathies</th>
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<tr>
<td>CACNA1A</td>
<td>Absence epilepsy + EA</td>
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<td>CHRNA4</td>
<td>ADNFLE</td>
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<td>CHRN2B</td>
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<td>CHRNA2</td>
<td>ADNFLE</td>
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<td>EE</td>
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TREATMENT CONSEQUENCES?
Dravet syndrome with SCN1A mutation

SCN1A loss of function

⇒ Aggravation of sz by sodium channel blockers

(carbamazepine, oxcarbazepine, lamotrigine, …)
KCNQ2 encephalopathy

- Dominant negative effect
  - Potassium channel opener retigabine
SCN2A encephalopathy

• Some patients respond well to sodium channel blockers (study ongoing)
MMPSI with KCNT1 mutation


KCNT1 gain of function in 2 epilepsy phenotypes is reversed by quinidine.

Targeted Treatment of Migrating Partial Seizures of Infancy with Quinidine

David Bearden, MD,¹
Alanna Strong, PhD,²
Jessica Ehnot, PharmD,³
Marissa DiGiovine, MD,¹
Dennis Dlugos, MD, MSCE,¹ and
Ethan M. Goldberg, MD, PhD¹

Migrating partial seizures of infancy is an early onset epileptic encephalopathy syndrome that is typically resistant to treatment. The most common cause is a gain of function mutation in the potassium channel KCNT1. The antiarrhythmic drug quinidine is a partial antagonist of KCNT1 and hence may be a candidate drug for treatment of this condition. We report the case of a child with migrating partial seizures of infancy secondary to an activating mutation in KCNT1 treated with quinidine. Treatment with quinidine was correlated with a marked reduction in seizure frequency and improved psychomotor development.

ANN NEUROL 2014;00:000–000
ORIGIN OF ID IN EPILEPSIES DUE TO ION CHANNEL MUTATIONS
Dravet syndrome

° Clinical
  • No strict correlation seizure severity - outcome
  • Treatment change later in life => improvement cognition

° Functional

Focal Scn1a knockdown induces cognitive impairment without seizures.
Bender AC¹, Natola H, Ndong C, Holmes GL, Scott RC, Lenck-Santini PP.

• siRNA in basal forebrain adult rats for 4 days
**KCNQ2 encephalopathy**

- **Clinical**
  - No strict correlation seizure severity – outcome

- **Functional**
  - Normal hippocampal morphology, not hyperactive, no overt behavioral seizures
  - Impaired spatial learning
  - Reduced M-type K+ current and neuronal hyperexcitability


*Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior.*

Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D.
De novo mutations in patients with ID/autism without epilepsy
Epilepsy and ID

Seizures do not explain everything

=> Target cause not only symptom

=> New strategies for treatment development
Neurogenetics group - epilepsy

- Rik Hendrickx
- Tine Deconinck
- Jolien Roovers
- Tania Djémié
- Katia Hardies
- Arvid Suls
- Peter De Jonghe

SPECIAL THANKS TO:

Parents and patients with KCNQ2 mutations

All treating physicians of patients with KCNQ2 mutations

Contact: sarahweck@hotmail.com