Genetics in neonatal and infant onset epileptic encephalopathies

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Neonatal and infant onset epileptic encephalopathies - electroclinical Syndromes

- Ohtahara Syndrome
- Epilepsy of Infancy with Migrating Focal Seizures
- West syndrome
- Dravet syndrome
Next Generation Sequencing (NGS)

- NGS have led to a virtual explosion of gene identifications both in familial cases and in epileptic encephalopathies

- Targeted sequencing of specific genomic regions
  - Custom designs: panel with all known epilepsy genes
  - Whole exome sequencing
  - Whole genome sequencing

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Targeted epilepsy panel

65 genes
52/500 patients (10%)

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

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Targeted epilepsy panel

- Mutations in 6 (SCN1A, CDKL5, STXBP1, CHD2, SYNGAP1, SCN2A) most common mutated genes in only 1-2% of cohort

- No mutations were seen in nine other known genes (ARX, FOXG1, KCNT1, MECP2, PLCB1, SLC25A22, SLC2A1 (GLUT1), SPTAN1, ARHGEF9)

⇒ Genetic heterogeneity

- Extending phenotype
  - PNKP in patient with normal HC and in
  - SCN1A in epilepsy-aphasia phenotype

=> Phenotypic heterogeneity

Carvill et al., 2013

Figure 1 Pathogenic and likely pathogenic mutations identified in 500 cases of epileptic encephalopathies in new (red) and known (blue) disease-related genes.

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These findings support a clinical approach to genetic diagnosis that employs large gene panels or whole exome sequencing, as it will remain difficult and expensive to determine *a priori* the causative gene in a given patient.
Genetic heterogeneity

Genes involved in epileptic encephalopathies November 2014

<table>
<thead>
<tr>
<th>ALG13</th>
<th>GABBR2</th>
<th>HCN1</th>
<th>RYR3</th>
<th>SPTAN1</th>
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<tr>
<td>ARHGEF9</td>
<td>GABRA1</td>
<td>IQSEC2</td>
<td>SCN1A</td>
<td>STXBP1</td>
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<td>GABRB3</td>
<td>KCNB1</td>
<td>SCN2A</td>
<td>STX1B</td>
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<td>CDKL5</td>
<td>GNAO1</td>
<td>KCNQ2</td>
<td>SCN8A</td>
<td>ST3GAL3</td>
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<td>CHD2</td>
<td>GRIN1</td>
<td>KCNT1</td>
<td>SCN1B</td>
<td>SYNGAP1</td>
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<td>DNM1</td>
<td>GRIN2A</td>
<td>PCDH19</td>
<td>SLC2A1</td>
<td>SZT2</td>
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<td>FASN</td>
<td>GRIN2B</td>
<td>PLCB1</td>
<td>SLC25A22</td>
<td>TBC1D24</td>
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<tr>
<td>FOXG1</td>
<td>HDAC4</td>
<td>PNKP</td>
<td>SLC35A2</td>
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</tr>
</tbody>
</table>
Electroclinical Syndromes

- Ohtahara Syndrome
- Epilepsy of Infancy with Migrating Focal Seizures
- West syndrome
- Dravet syndrome
Case 1

- 4 year old girl with severe ID and intractable epilepsy
- Born at term with birth weight 4630 g and head circumference 38 cm
- The neonatal period was complicated by poor feeding
- Unspecified stereotypic jerks in the lower extremities were noticed. At the age of 2 ½ months the child developed epileptic spasms-like episodes
- 3 m.o.a she had frequent myoclonic jerks in her legs and focal seizures with secondary generalization
- Developmentally delayed with no eye contact. She was opisthotonic and had a spastic muscle tone most severely in the lower extremities
- Dysmorphic features: broad nasal bridge, deep philtrum, high palate, and her HC was 39 cm (-25 percentile)
Case 1

- EEG at 3 m.o.a showed burst-suppression pattern
- MRI at 3 m.o.a showed asymmetrical subarachnoidal space in temporal region
- Developed multiple seizure-types including myoclonic jerks, focal seizures with secondary generalisation, epileptic spasms and GTCS
- Fluctuating seizure frequency varying from 10-15 seizures per day to a few per month
- From 6 m.o.a. she had stereotypic wave movements of hands, and at the age of 4 years she developed series of non-epileptic spasm like episodes
- Oxygen dependent due to multiple pneumonias, food aspirations and asthma
- Profound intellectual disabled without any spoken language
- Spastic tetraparesis
Ohtahara Syndrome

- Ohtahara syndrome is one of the most severe and earliest forms of epilepsy.
- Brain malformations or metabolic disorders are often associated, but other cases remain etiologically unexplained.
- Genes in Ohtahara syndrome:
  - **STXBP1**
  - **ARX** – only boys
  - **SCN2A** – ~10 patients described
  - **KCNQ2** – neonatal onset of intractable seizures with a prominent tonic component, burst-suppression pattern, early MRI: characteristic hyperintensities in the basal ganglia and thalamus
  - **GNAO1** – 5 patients
  - **SLC25A22** – few recessive families
  - **(KCNT1)** – only reported in 1 case (homozygous mutation)
Case 1

- *De novo* GNAO1 mutation c.692A>G; p.Tyr231Cys

- GNAO1 encodes an alpha subunit of the heterotrimeric guanine nucleotide-binding proteins (G proteins), a large family of signal-transducing molecules

- Mice lacking Gαₒ show multiple neurological abnormalities, including generalized tremor, occasional seizures, severe motor-control impairment, hyperalgesia, and behavioral abnormalities with early postnatal lethality

- Mutations in GNAO1 have recently been described in four patients with severe early infantile epileptic encephalopathies

- New Ohtahara gene
Case 2

- 2 year old boy with severe ID and intractable epilepsy
- Neonatal period was complicated by poor feeding, hypotonia and onset of focal seizures day 1
- 5 months old he developed epileptic spasms
- EEG: hypsarrythmia
- Currently, up to 20 focal seizures pr day
- EEG: multifocal discharges
- MRI: normal
- Severely hypotonic, profound ID

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Idiopathic west syndrome

- West syndrome is characterised by the combination of clustered spasms and hypsarrhythmia on an EEG and delayed brain development or regression.

- Genetic heterogeneity
  - STXBP1
  - CDKL5 - girls
  - ARX - boys
  - DNM1
  - SLC25A22
  - SPTAN1
  - PLCB1
  - PNKP
  - FOXG1
  - RYR3
  - ALG13
  - GRIN1
  - GRIN2B
  - KCNB1
  - SPTAN1
  - SLC2A1
  - GABRB3
**GABRB3**

- **GABRB3**: c.767T>A, p.Leu256Gln *de novo*

- Encodes one of the subunits of a multi-subunit chloride channel that serves as the GABA receptor.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Trio</th>
<th>Phenotype</th>
<th>Gender</th>
<th>Age at onset</th>
<th>Initial seizure type</th>
<th>Additional seizure types</th>
<th>EEG features</th>
<th>MRI</th>
<th>Prior to seizure onset</th>
<th>Regression?</th>
<th>Dev. at last review</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABRB3</td>
<td>dr</td>
<td>IS</td>
<td>F</td>
<td>5 mo</td>
<td>IS</td>
<td>IS only</td>
<td>hypsarrhythmia, that evolved from MFD</td>
<td>normal</td>
<td>normal</td>
<td>unk</td>
<td>unk</td>
<td>myoclonic spells as well as classic spasms</td>
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<tr>
<td>GABRB3</td>
<td>gs</td>
<td>LGS</td>
<td>F</td>
<td>10 mo</td>
<td>FDS, staring spells</td>
<td>FDS, GTC w SE</td>
<td>8 hertz background</td>
<td>normal</td>
<td>normal to mild delay</td>
<td>unk</td>
<td>severe delay w/ 20 words at 4 yrs</td>
<td>mixed EE with left temporal features</td>
</tr>
<tr>
<td>GABRB3</td>
<td>jw</td>
<td>LGS</td>
<td>M</td>
<td>10 mo</td>
<td>IS</td>
<td>GTC, atypical absence, myoclonic, atonic</td>
<td>bursts of 2 Hz sharp-SW complexes</td>
<td>normal</td>
<td>mild delay</td>
<td>no</td>
<td>Adaptive scores &lt;20, could not measure IQ</td>
<td>ADHD, Mood lability with impulsive behavior</td>
</tr>
<tr>
<td>GABRB3</td>
<td>jr</td>
<td>LGS</td>
<td>M</td>
<td>10 mo</td>
<td>IS</td>
<td>Febrile, GTC, tonic, atypical absence, myoclonic, atonic</td>
<td>Generalized 2 Hz bursts</td>
<td>small acute infarct in splenium of CC in 2010</td>
<td>delayed, did not roll over by 10 months</td>
<td>no</td>
<td>FSIQ=44, VABS=68</td>
<td>ADHD, impulsive, sleeping difficulties</td>
</tr>
</tbody>
</table>
Case 3

- 3 year old boy with profound intellectual disability and intractable epilepsy
- Onset of hemiclonic seizures 3 months of age
- Later he developed spasms, FDS, asymmetric tonic
- Several episodes of status epilepticus
- Interictal EEG showed multi-focal spikes and slowing
- Ictal EEG revealed shifting foci of ictal onset
- Early development was normal – regression after seizure onset - profound intellectual disability
- Acquired microcephaly
- Choreriform movements
- MRI was normal

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Epilepsy of Infancy with Migrating Focal Seizures

- Presents in infancy and is associated with near constant polymorphous pharmaco-resistant seizures that migrate throughout the brain and are associated with severe developmental delay

- Genes in EIMFS
  - KCNT1
  - SCN1A
  - TBC1D24
  - PLCB1
  - (CACNA1A?)
Case 4

- 3 year old boy with ID and intractable epilepsy
- Normal pregnancy, sectio 38+0, Apgar 9/10
- 3 m.o.a – hemiclonic FS (lasting 1 hour)
- 7 m.o.a – first afebrile GTC seizure
- Hemiclonic seizures, GTCS, FDS, atypical absences, tonic seizures
- Several episodes of febrile status
- Delayed development after seizure onset
- EEG: initially normal
- EEG 24 m.o.a – multifocal
- MRI normal
- Currently, daily seizures, severe ID, ADHD
Dravet syndrome

• > 80% of Dravet patients carry a SCN1A mutation

• Among 819 SCN1A mutations Zuberi 2011
  – 49% missense: variable phenotype
  – 51% truncation: severe phenotype

• Nature of mutation affects severity of phenotype
  • Truncation have earlier mean onset:
    – Prolonged seizures 7 versus 9 months
    – Myoclonic seizures 16 versus 19 months
    – Atypical absences 19 versus 30 months
Dravet syndrome

- Genetic heterogeneity in remaining 20%
  - Recessive mutations in *SCN1B*
  - Heterozygous mutations in *PCDH19, HCN1, GABRA1, STXBP1, SCN8A, CHD2*
  - Rarely “typical” Dravet patients
PCDH19 spectrum

- Familial or sporadic
- Seizures onset 4 mo – 5 years (mean 12 mo)
- Seizures in clusters
- Epileptic encephalopathy or mild epilepsy
- Normal intellect to severe ID
- Normal behavior to severe autism
- Inter- and intrafamilial variability

- Compared to typical Dravet syndrome:
  - Later onset
  - Less frequent status epilepticus and myoclonic sz
  - Seizures in clusters
  - Better outcome
De novo mutations in HCN1 cause early infantile epileptic encephalopathy

- Hyperpolarization-activated cyclic nucleotide-gated channel 1
- Permeable for Na+ and K+

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Clinical: *HCN1*

<table>
<thead>
<tr>
<th>Family number</th>
<th>Subject origin</th>
<th>Sex</th>
<th>Base change</th>
<th>Amino acid change</th>
<th>Exon</th>
<th>Inheritance</th>
<th>Age at time of analysis (years)</th>
<th>Age at seizure onset (months)</th>
<th>Seizure types</th>
<th>Status epilepticus</th>
<th>Intellectual disability</th>
<th>MRI</th>
<th>Pharmacoresistance</th>
<th>Behavior and language</th>
<th>Other features</th>
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<tbody>
<tr>
<td>1</td>
<td>France</td>
<td>Female</td>
<td>c.140G&gt;T</td>
<td>p.Gly47Val</td>
<td>1</td>
<td>De novo</td>
<td>18</td>
<td>4</td>
<td>FS, TCS, absence, focal, myoclonic</td>
<td>Yes</td>
<td>Moderate to severe</td>
<td>NA</td>
<td>Yes</td>
<td>Yes</td>
<td>Absence of language</td>
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<td>2</td>
<td>Italy</td>
<td>Female</td>
<td>c.299C&gt;T</td>
<td>p.Ser100Phe</td>
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<td>De novo</td>
<td>16</td>
<td>8</td>
<td>FS, TCS, absence, focal, myoclonic</td>
<td>Yes</td>
<td>Moderate to severe</td>
<td>Normal</td>
<td>Yes</td>
<td>Yes</td>
<td>Autistic features</td>
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<tr>
<td>3</td>
<td>France</td>
<td>Female</td>
<td>c.814T&gt;C</td>
<td>p.Ser272Pro</td>
<td>2</td>
<td>De novo</td>
<td>12</td>
<td>13</td>
<td>FS, CS, focal, absence</td>
<td>Yes</td>
<td>Severe</td>
<td>Normal</td>
<td>Yes</td>
<td>Yes</td>
<td>Behavioral disturbances, autistic features</td>
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<tr>
<td>4</td>
<td>The Netherlands</td>
<td>Male</td>
<td>c.835G&gt;T</td>
<td>p.His279Tyr</td>
<td>2</td>
<td>De novo</td>
<td>15</td>
<td>8</td>
<td>FS (atypical), CS, TCS, absence, myoclonic</td>
<td>No</td>
<td>Mild</td>
<td>Normal</td>
<td>Yes</td>
<td>Yes</td>
<td>Behavioral disturbances, autistic features</td>
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<td>5</td>
<td>France</td>
<td>Female</td>
<td>c.890G&gt;C</td>
<td>p.Arg297Thr</td>
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<td>De novo</td>
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<td>4</td>
<td>FS, focal, absence</td>
<td>Yes</td>
<td>Moderate to severe</td>
<td>Normal</td>
<td>Yes</td>
<td>Yes</td>
<td>Behavioral disturbances, autistic features</td>
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<td>6</td>
<td>France</td>
<td>Female</td>
<td>c.1201G&gt;C</td>
<td>p.Asp401His</td>
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<td>De novo</td>
<td>18</td>
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<td>FS, TCS, absence, focal, myoclonic</td>
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<td>Moderate to severe</td>
<td>Normal</td>
<td>Yes</td>
<td>Yes</td>
<td>Polypagia</td>
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</table>

FS, febrile seizure; TCS, tonic-clonic seizure; CS, clonic seizure; MRI, magnetic resonance imaging; ADHD, attention deficit hyperactivity disorder; NA, unavailable.
## Phenotypic heterogeneity

### Severity

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>Syndrome</th>
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<tbody>
<tr>
<td>GABRA1</td>
<td>IGE</td>
<td>Dravet</td>
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<tr>
<td>GABRB3</td>
<td>FS</td>
<td>EE</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>BFNS</td>
<td>Neonatal EE</td>
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<tr>
<td>KCNT1</td>
<td>ADNFLE</td>
<td>EIMFS</td>
</tr>
<tr>
<td>SCN1A</td>
<td>FS, GEFS+</td>
<td>Dravet syndrome</td>
</tr>
<tr>
<td>SCN2A</td>
<td>BFNIS</td>
<td>EE</td>
</tr>
<tr>
<td>SCN8A</td>
<td>BFIS/ICCA</td>
<td>EE</td>
</tr>
<tr>
<td>SCN1B</td>
<td>GEFS+</td>
<td>Dravet</td>
</tr>
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</table>
Phenotypic heterogeneity

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.


Family 2: KCNT1 c.1193G>A; p.Arg398Gln

Learning difficulties
MMFSI
ADNFLE
Multifocal epilepsy

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Danish gene panel

- Making a genetic diagnosis in a patient with EE can be challenging as there is both genetic heterogeneity for a given epilepsy syndrome and phenotypic heterogeneity for a specific gene.

- A gene panel targeting 45 epilepsy genes was developed for next generation sequencing.

- Genes associated with childhood epilepsies mainly epileptic encephalopathies.
Targeted next generation sequencing as a diagnostic tool

- We analyzed this panel on a cohort of 125 patients

- The majority of the patients in this cohort had **epileptic encephalopathies (EE)** and a few patients suffered from milder epilepsy syndromes such as GEFS+, ADNFLE, familiar focal epilepsy, benign familiar neonatal/infantile seizures
Findings

- We identified a presumed disease-causing mutation in 31 of 125 patients
  
  - 11 SCN1A (1 GEFS+, 10 Dravet (adults 19-52 years))
  - 4 STXBP1 (4 EE)
  - 3 SCN2A (EE)
  - 2 CDLK5 (EE)
  - 2 KCNQ2 (EE)
  - 1 KCNT1 (ADNFLE)
  - 1 CACNA1A (MMPSI)
  - 1 GABRA1 (GEFS+/Dravet)
  - 1 GABRB3 (EE)
  - 1 PNPO (EE)
  - 1 GNAO1 (EE)
  - 1 STX1B (EE)
  - 1 SCN8A (EE)
  - 1 CHD2 (EE)
  
  - 3 inherited mutation (milder phenotypes), 1 compound heterozygous mutation, **27 heterozygous de novo mutations**
  
  - Array CGH: *de novo* copy number variants (CNVs) account for up to ~8% of cases
  
  - In at least 10 of the patients the genetic diagnosis has lead to changes in the medication

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Summary

- *De novo* mutations many different genes can cause epileptic encephalopathies

- Making a genetic diagnosis in a patient with EE can be challenging as there is both genetic heterogeneity for a given epilepsy syndrome and phenotypic heterogeneity for a specific gene

- We have developed a rapid and cost-efficient screening panel for the analysis of the genetic basis of severe childhood epilepsies

- We were able to find a disease-causing genetic variation in 24% of the analyzed patients

- This approach is critical to identify additional patients with mutations in genes where a single *de novo* mutation is identified by e.g. exome sequencing approaches

- To determine overall mutation frequency in a given phenotype

- To describe genotype-phenotype correlations

- With sufficient number of patients identified per gene, personalized medicine and large-scale compound screening are on the horizon, which will eventually result in new therapeutic strategies for the patients

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