The importance of genetic diagnosis in early onset epilepsy
Childhood Epilepsy Masterclass
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Outline

• The genetic basis of early onset epilepsy
• Emerging/novel phenotypes
• Novel diagnostic approaches- what can we do?
• When/how does a genetic diagnosis make a difference?
• What does the future hold?

How much of early onset epilepsy is genetic??

Important genetic diagnoses- metabolic disorders

• SLC2A1
• Antiquitin

What do we mean by “genetic”? 

• Monogenetic
• Copy number variation
• Recognisable genetic syndromes
• Chromosomal disorders
• Polygenic/complex
• Multifactorial- 2 hits

The genetic basis of early onset epilepsy

• Early onset monogenic (or CNV-related) epileptic encephalopathies where seizures are the prominent feature and imaging is normal
• Syndromes with severe developmental delay and epilepsy amongst other features
How to classify genetic early onset epilepsy?

- Electroclinical syndrome
- OMIM classification - EIEE1-18

Seizure types
- Tonic spasms
- Myoclonic
- Focal +/- autonomic features, migrating spasms
- Febrile hemi-clonic

Other features
- DD
- Dyskinesia
- Evolution to WS
- Movement disorder
- Later gait disorder

EEG
- BS
- Migrating foci
- Hyps
- Normal initially

MRI
- MCD
- Hypomyelination
- May be normal
- Atrophy
- Delayed/hypo myelination
- WMH
- Variety of structural malformations
- Normal

Aetiology
- Structural
- Metabolic
- Genetic
- Multiple
- Genetic causes:
  1. ARX
  2. CDKL5
  3. SLC25A22
  4. STXBP1
  5. SPTAN1
  6. SCN1A
  7. KCNQ2
  8. ARHGEF9
  9. PCDH19
  10. PNKP
  11. SCN1A
  12. ARX
  13. CDKL5
  14. SLC25A22
  15. STXBP1
  16. SPTAN1
  17. SCN1A
  18. KCNQ2
  19. ARHGEF9
  20. PCDH19

The complexities of diagnosis in early onset epilepsy - phenotypic pleiotropy

- Dravet syndrome
- West syndrome
- SCN1A
- MPSI
- Non-specific EIEE

The complexities of diagnosis in early onset epilepsy - genetic heterogeneity

- ARX
- CDKL5
- SLC25A22
- STXBP1
- SPTAN1
- SCN1A
- KCNQ2
- ARHGEF9
- PCDH19

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- SLC25A22
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- SPTAN1
- SCN1A
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- ARHGEF9
- PCDH19
Distinct genotype-phenotype correlations in early onset epilepsy: Dravet syndrome

• 1 in 40,000 children
• Febrile or afebrile generalised or hemiconvulsions starting in the first year of life
• Seizure evolution - intractable generalised (myoclonic or atonic seizures, atypical absences) and focal seizures
• Normal early development, subsequent developmental delay and behavioral problems
• Evolving spasticity and crouch gait

Dravet syndrome

• 70-80% sequencing mutations in SCN1A
  40% truncating
  40% missense
  Remainder splice site
• 2-3% Intragenic/whole gene deletions of SCN1A +/- other contiguous genes (12.5% of mutation-negative DS)

Difficulties in genetic diagnosis in Dravet

• Usually de novo (90-95%) although often a family history of FS/epilepsy
• Germline/somatic mosaicism in ~7% DS families
• Explanation for intrafamilial phenotypic variability
• Lack of consistent genotype-phenotype correlation
• SCN1A negative DS patients: PCDH19, GABRG2, SCN1B

What is the impact of a genetic diagnosis in DS?

Clinician’s views
• Additional investigations were avoided in 67%
• 69% altered treatment
• 74% helped medication choice.
• Improved seizure control in 42% after medication change
• 50% avoided inappropriate drugs

Parent’s views:
• 87% helpful in giving an explanation
• 55% led to a change in treatment
• 69% of this group fewer seizures
• 34% improvement in developmental progress
• 41% other benefits: improved access to therapies/respite care/change of goals.

STXB1- not just Ohtahara syndrome?

• First described in a deletion in OS and then in larger OS cohorts - ~30% hit rate
• De novo, heterozygous
• Spectrum of phenotypes from West syndrome to ID without seizures - limited number of cases
• Movement disorder common - tremor, dyskinesia

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What is the impact of a genetic diagnosis in DS?

- Earlier diagnosis - better treatment of status
- Avoidance of carbamazepine
- Use of appropriate medications
- Impact on long term cognitive outcome

Emerging genotype-phenotype correlations in early onset epilepsy:

- PCDH19
- KCNQ2
- CDKL5
- CHD5
- KCNT1

Case example

- 12 months
- Explosive onset of epileptic encephalopathy with developmental regression
- Ix (MRI, CSF, autoimmune aetologies, mitochondrial disease) negative

- Half-sister aged 4 years: clusters of GTC from 13 months, moderate learning difficulties and ASD
- Mum - clusters of seizures from 13 months to 14 years of age

Key features of protocadherin 19 (PCDH19) related epilepsy

- Seizures beginning by age 3 years
- 67% of affected females have learning difficulties of borderline intellect.
- Early development varies from normal to abnormal; developmental regression commonly occurs with seizure onset.
- Obsessive features in 33%, aggressive behaviour in 26%, ASD in 22%

PCDH19 related epilepsy

- If girl with Dravet syndrome SCN1A negative, then test PCDH19
- Families with only girls affected
  - Unusual: X-linked - females affected, male carriers
- Cellular interference
- Spectrum of severity in females
- Seizures may burn-out in teenage years
- Clobazam can be helpful
- Important diagnosis due to counselling implications
KCNQ2 related EIEE

- 8/80 with EIEE of unknown cause had KCNQ2 mutations
- Seizure onset first week of life
- Tonic seizures with autonomic features
- EEG – SBS or multifocal
- MRI- basal ganglia hyperintensities, some transient
- Retigabine

CDKL5 – not just an early onset Rett syndrome

- Infantile spasms by 5th month
- Peculiar seizure pattern with a tonic-vibratory contraction, followed by a clonic phase with series of spasms, gradually translating into repetitive distal myoclonic jerks
- Poor gaze, hand stereotypes and bruxism
- But 30% will walk and epilepsy may resolve in 50%
- Rett-like- consider MECP2, FOXG1, WDR45

CHD2 related epilepsy

- Identified as candidate gene within CNV
- In 500 EIEE cases, 1.2% had de novo het mutations in CHD2
- Onset 12 months -3 years (median 18 months)
- Photosensitive ++
- Myoclonic and atonic seizures amongst others
- 7/9 SCN1A-negative Dravet syndrome cases in recent series

Migrating Partial Seizures of Infancy (MPSI)

- Onset before 6 months
- Normal development before seizure onset
- Migrating focal motor seizures at onset
- Characteristic semiology & EEG
- Multifocal seizures - intractable to conventional AEDs
- No identified aetiology
- Profound psychomotor delay
- Rare ~100 cases reported to date

Materials and Methods

- Whole exome sequencing (Illumina/Hi-Seq) (n=5)
- Direct Sanger sequencing (n=4)
- Segregation studies with parental DNA
Results

- 3 patients identified with mutations in KCNT1
- ~30% hit rate
- 2 patients with previously identified mutation
  - c.2800G>A; p.Ala934Thr
- 1 patient with novel mutation
  - c.811G>T; p.Val271Phe

Clinical features of KCNT1 positive patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at seizure onset (weeks)</th>
<th>MRI</th>
<th>Novel EEG findings</th>
<th>Novel clinical features</th>
<th>Initial neurology exam</th>
<th>Later neurology exam</th>
<th>Age at death</th>
<th>Age at seizure onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Atrophy</td>
<td>MH with WMI</td>
<td>MRS low NAA</td>
<td>GI dysmotility</td>
<td>Asym. hypotonia</td>
<td>5 yrs 4 months</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Atrophy</td>
<td>MH with WMI</td>
<td>ECDT, ataxia, hypotonia</td>
<td>Hypotonia, peripheral spasticity</td>
<td>5 yrs</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
<td>MRS low NAA</td>
<td>Normal</td>
<td>Normal</td>
<td>19 months</td>
<td>2</td>
</tr>
</tbody>
</table>

The genetic basis of MPSI

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Exon</th>
<th>Gene Function</th>
<th>Inheritance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNT1</td>
<td>8</td>
<td>8</td>
<td>Na⁺ activated K⁺ channel subunit</td>
<td>Denovo autosomal dominant</td>
<td>Barcia et al 2012, McTague et al 2013</td>
</tr>
<tr>
<td>TBC1D24</td>
<td>2</td>
<td>2</td>
<td>GTPase-activating protein regulation of membrane trafficking</td>
<td>Autosomal recessive</td>
<td>Milh et al 2013</td>
</tr>
<tr>
<td>PLCB1</td>
<td>1</td>
<td>1</td>
<td>G-protein coupled enzyme, IP3/DAG formation, intracellular transduction of many extracellular signals</td>
<td>Autosomal recessive</td>
<td>Poduri et al 2012</td>
</tr>
<tr>
<td>SCN1A</td>
<td>3</td>
<td>3</td>
<td>α subunit of voltage gated sodium channel evoking neuronal action potentials</td>
<td>Denovo autosomal dominant</td>
<td>Carranza et al 2011, Freilich et al 2011</td>
</tr>
<tr>
<td>SCN2A</td>
<td>1</td>
<td>1</td>
<td>α subunit of voltage gated sodium channel</td>
<td>Denovo autosomal dominant</td>
<td>Dhamija et al 2013</td>
</tr>
<tr>
<td>SLC25A22</td>
<td>1</td>
<td>1</td>
<td>Mitochondrial glutamate transport</td>
<td>Autosomal recessive</td>
<td>Poduri et al 2011</td>
</tr>
</tbody>
</table>

Copy number variation in EIEE

- Micro-deletion syndromes associated with epilepsy e.g Angelman’s (15q11.2-q13),
- Meford et al: 315 patients with infantile and childhood epileptic-7.9% to have CNVs, half of which were clearly pathogenic.
- well recognised epilepsy-associated genes such as ARX
- duplications of 16p11.2- previously reported in migrating partial seizures in infancy.
- Microarray has a good yield in the investigation of EIEE
New approaches to genetic diagnosis - gene panel at GOSH

- Often difficult to predict genotype from phenotype
- Considerable cost to NHS to sequentially screen genes
- Develop a multiple gene panel
  - In collaboration with diagnostic genetic colleagues
  - Simultaneous screening of 29 genes
  - Pilot study
  - Translation into clinical practice

Next gen gene panels

1. Digest DNA
   - Sample is fragmented using restriction enzymes
2. Hybridize probes
   - Probe library is added and hybridized to the targeted fragments
   - Making them form a halo shape
3. Purify and ligate targets
   - Probe/Fragment hybrids are retrieved with magnetic streptavidin beads
   - Circular molecules are then closed by ligation
4. Amplify targeted fragments
   - Only circular DNA targets are amplified
   - Sample barcodes are introduced
   - Final product is ready for sequencing

Materials and Methods

- 48 children
- Early onset seizures, severe global developmental delay
- Pre-screening with microarray/methylation studies (Angelman’s syndrome)
- Copy number variants - exon-level aCGH (Roche NimbleGen)
- Point mutations – next generation sequencing methods (Haloplex custom capture / Illumina)

Data analysis

- NextGENe software (Softgenetics)
- Array data: CGH Fusion (InfoQuant)
- Point mutations: missense, nonsense, insertions, deletions
  - (i) Confirmed by traditional Sanger sequencing
  - (ii) Familial segregation studies
- Single exon-level copy number variants

Confirmed by Multiplex ligation-dependent probe amplification (MLPA) or quantitative PCR

RESULTS

- Mutations identified in 5 patients (~10%)
- Data quality high: >30x sequence data coverage, 90% of targeted coding bases, no false positive calls made

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Presentation</th>
<th>Gene</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Microcephaly 5 weeks GDD</td>
<td>CDKL5</td>
<td>49kb Duplication Exons 3-7</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Microcephaly GDD</td>
<td>MECP2</td>
<td>Nonsense mutation</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Microcephaly GDD</td>
<td>MECP2</td>
<td>3kb deletion Exon 4</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Microcephaly GDD Dyskinesia</td>
<td>SLC39A6</td>
<td>c.808delA</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>Microcephaly GDD</td>
<td>EHMT1</td>
<td>c.1596delC</td>
</tr>
</tbody>
</table>
PANEL ROUND 2

- expanded gene list
- improved coverage
- current hit rate 10-20%
- mutations identified in FOXG1, UBE3A, KCNT1, CDKL5, SCN2A to date

Gene panels in childhood epilepsy - published experience

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>No. of genes</th>
<th>Technique</th>
<th>Hit rate</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemke 2012</td>
<td>33 patients with childhood onset epilepsy of varying phenotypes</td>
<td>265</td>
<td>Agilent SureSelect SOLiD4</td>
<td>48%</td>
<td>Vary from non-screened Dravet's cases to non-specific MR with seizures</td>
</tr>
<tr>
<td>Carvill 2013</td>
<td>500 patients with childhood onset epilepsy</td>
<td>65 (19 known, 46 candidate)</td>
<td>MIPs/HiSeq</td>
<td>10%</td>
<td>INFANTILE SPASMS: 5%, OHTAHARA: 50%</td>
</tr>
<tr>
<td>Kodera 2013</td>
<td>53 patients with early onset epilepsy</td>
<td>35 (30 known, 5 candidate)</td>
<td>Agilent SureSelect Illunina</td>
<td>22%</td>
<td>High coverage and long reads able to detect small CNVs</td>
</tr>
</tbody>
</table>

Whole exome in early onset epilepsy - published experience

<table>
<thead>
<tr>
<th>Author</th>
<th>No of subjects</th>
<th>Phenotype</th>
<th>Hit rate</th>
<th>Novel genes identified</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veeramah 2013</td>
<td>10 triomes</td>
<td>3 WS, 2 DS, remainder non-specific</td>
<td>7/10 de novo causal mutations</td>
<td>KCNQ5, GLO4, ARHGEF15</td>
<td>Small cohort Functional validation</td>
</tr>
<tr>
<td>Epi4K consortium 2013</td>
<td>264 triomes</td>
<td>Infantile spasms, Lennox-Gastaut syndrome</td>
<td>29 patients with de novo mutations</td>
<td>GABRB3, ALG13</td>
<td>Little info on phenotypes No functional validation</td>
</tr>
</tbody>
</table>

Gene panel or whole exome?

Pathways in EIEE

- Abnormal synaptic function
- Stabilisation of membrane proteins
- Solute transport (transient)
- Transcription (DNA repair)
- DNA repair
- Channelopathies
- Severe early onset epilepsy
Conclusions

• A genetic diagnosis may limit investigations and direct treatment
• Psychological/goal-setting benefits
• Benefits of a label
• Advancing understanding of common pathways/influence on networks
• Development of novel treatments
• Caveats: limited ability to prognosticate, difficulties of diagnosis

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Please contact us regarding any patients with EIEE

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