## COI Disclosure

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Honoraria/Expenses</th>
<th>Consulting/Advisory Board</th>
<th>Funded Research</th>
<th>Royalties/Patent</th>
<th>Stock Options</th>
<th>Ownership/Equity Position</th>
<th>Employee</th>
<th>Other (please specify)</th>
</tr>
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<tbody>
<tr>
<td>Invitae</td>
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</tbody>
</table>
Multi-gene sequencing panel is a useful first test with a high diagnostic yield in childhood epilepsy

Darlene Riethmaier, MS, CGC
Invitae, San Francisco, USA
Genetics of epilepsy

- Epilepsy is defined as the occurrence of two or more unprovoked seizures separated by at least 24 hours
  - Affects ~65 million people worldwide (ILAE/WHO)
- Several forms are classified, affecting a plethora of biological pathways
  - Genetically and clinically heterogeneous
  - >50% of etiologies are genetic*
- Research efforts (Epi4K) are identifying novel genes and illuminating new mechanisms
- Utility of genetic testing:
  - Molecular diagnosis
  - Clinical correlation (syndromic or not? prognosis?)
  - Recurrence risk
  - Clinical management

Genetic testing for epilepsy

- Traditionally, single-gene sequencing of ion channel genes (SCN1A, CHRNA4, KCNQ2, etc.) is expensive and slow.
- Using next-generation sequencing for multi-gene panel testing rapidly expanded testing in the last 5 years lead to improved diagnostic yield.
- Whole exome sequencing is now used in diagnostic odysseys.

Goals of our study:
- Describe testing results from a cohort of 2,000 unrelated individuals.
- Explain the mutation spectrum, including prevalence of CNVs.
- Point out proportion of “actionable” cases.
- Compare diagnostic yield to exome studies.
Invitae epilepsy panel testing results

- Used a next-generation sequencing multi-gene panel consisting of 105 to 185 genes
- Patients were referred for testing for all genes or a subset (e.g., infantile epileptic encephalopathy or Rett/Angelman spectrum)
- Analysis included simultaneous detection of sequence variants and exon-level copy number variants (deletions and duplications)
  - NGS-based copy number calling is highly sensitive
  - Positive findings were confirmed by traditional exon-focused array CGH

<table>
<thead>
<tr>
<th>Panel</th>
<th># Patients</th>
<th>Positive yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full panel</td>
<td>1775</td>
<td>20% (MDx 16%)</td>
</tr>
<tr>
<td>Early infantile epileptic encephalopathy</td>
<td>47</td>
<td>19% (MDx 19%)</td>
</tr>
<tr>
<td>Rett / Angelman spectrum (syndromic epilepsy)</td>
<td>84</td>
<td>25% (MDx 24%)</td>
</tr>
</tbody>
</table>

MDx = definitive molecular diagnosis
## Genes with the highest positive results

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th># individuals</th>
<th>P/LP variants</th>
<th>MDx</th>
<th>% MDx</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN1A</td>
<td>AD</td>
<td>1866</td>
<td>49</td>
<td>49</td>
<td>2.6%</td>
</tr>
<tr>
<td>MECP2</td>
<td>XL</td>
<td>1860</td>
<td>23</td>
<td>23</td>
<td>1.2%</td>
</tr>
<tr>
<td>PRRT2</td>
<td>AD</td>
<td>1776</td>
<td>20</td>
<td>20</td>
<td>1.1%</td>
</tr>
<tr>
<td>TPP1</td>
<td>AR</td>
<td>1776</td>
<td>20</td>
<td>7</td>
<td>0.4%</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>AD</td>
<td>1854</td>
<td>19</td>
<td>19</td>
<td>1.0%</td>
</tr>
<tr>
<td>STXBP1</td>
<td>AD</td>
<td>1906</td>
<td>15</td>
<td>15</td>
<td>0.8%</td>
</tr>
<tr>
<td>DEPDC5</td>
<td>AD</td>
<td>1775</td>
<td>11</td>
<td>11</td>
<td>0.6%</td>
</tr>
<tr>
<td>ALDH7A1</td>
<td>AR</td>
<td>1857</td>
<td>11</td>
<td>3</td>
<td>0.2%</td>
</tr>
<tr>
<td>CDKL5</td>
<td>XL</td>
<td>1906</td>
<td>11</td>
<td>11</td>
<td>0.6%</td>
</tr>
<tr>
<td>POLG</td>
<td>AR</td>
<td>1795</td>
<td>10</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>PCDH19</td>
<td>XL</td>
<td>1822</td>
<td>10</td>
<td>10</td>
<td>0.5%</td>
</tr>
<tr>
<td>SYNGAP1</td>
<td>AD</td>
<td>1822</td>
<td>8</td>
<td>8</td>
<td>0.4%</td>
</tr>
<tr>
<td>WWOX</td>
<td>AR</td>
<td>1822</td>
<td>8</td>
<td>2</td>
<td>0.1%</td>
</tr>
<tr>
<td>SCN2A</td>
<td>AD</td>
<td>1859</td>
<td>8</td>
<td>8</td>
<td>0.4%</td>
</tr>
<tr>
<td>KIAA2022</td>
<td>XL</td>
<td>988</td>
<td>4</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>BRAT1</td>
<td>AR</td>
<td>1015</td>
<td>4</td>
<td>1</td>
<td>0.1%</td>
</tr>
<tr>
<td>SLC2A1</td>
<td>AD</td>
<td>1830</td>
<td>7</td>
<td>7</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

- ** Syndromic 17% **
- ** Syndromic and EIEE 10% **
- ** EIEE 28% **
- ** Everything Else 23% **
- ** Treatable 22% **
Spectrum of variants identified

- Sequence variants included single-nucleotide changes, small indels, large indels, ARX trinucleotide expansion.
- Copy number variants included exonic deletions, exonic duplications, cytogenetic changes.
- Majority of pathogenic variants were not missense changes, in contrast to VUS.
- VUS in AD high penetrance genes, compound heterozygous or homozygous variants in AR genes have a chance of resolution
- A majority of VUS cannot be resolved because they are single variants in AR genes or in AD reduced penetrance genes
Examples of positive copy number variants

CDKL5

STXBP1
Expansion of 1st poly-alanine track in exon 2 is associated with EIEE or West syndrome

- Detectable by optimized NGS

An example case observed:

- 6 month old with infantile spasms
- 21 base duplication (expansion from 16 to 23 alanine residues) in the alanine repeat
Rare Mosaic Variants Identified

- Mosaic variants identified in **CDKL5**, **FRRS1L**, **TSC1** and **TSC2**
Rare Dual Molecular Diagnoses

- De novo SNVs found in both *STXBP1* and *SYNGAP1*
  - One-month-old infant with a history of seizures beginning in first days of life
  - Normal brain MRI, normal metabolic work-up to date

- Deletion of both *SCN1A* and *SCN9A*, suggesting a chromosome 2 abnormality
  - Epilepsy, unspecified, not intractable, without status epilepticus

- Pathogenic variants identified in *CDKL5*; Likely Pathogenic variant identified in *PRRT2*
  - 4 year old with intractable epilepsy and developmental delay

- These genes are all high penetrance
High frequency of mutations in new genes (*DEPDC5*, *PRRT2*)

Higher than suspected prevalence of “rare epilepsies”
  - e.g., *PCDH19*, *SYNGAP1*, *PRRT2*, *DEPDC5*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pathogenic variants</th>
<th>% of all pathogenic results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>PRRT2</em></td>
<td>20</td>
<td>4.8%</td>
</tr>
<tr>
<td><em>DEPDC5</em></td>
<td>11</td>
<td>2.6%</td>
</tr>
<tr>
<td><em>PCDH19</em></td>
<td>10</td>
<td>2.4%</td>
</tr>
<tr>
<td><em>SYNGAP1</em></td>
<td>8</td>
<td>1.9%</td>
</tr>
</tbody>
</table>
Results impacting treatment

- 22% of all molecular diagnoses were found in these genes
- Panel testing provides a fast turn-around time (2-3 weeks) and therefore helps manage treatment in these rare cases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disorder</th>
<th>Inheritance</th>
<th>Individuals tested</th>
<th>MDx</th>
<th>% MDx</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN1A</td>
<td>Dravet syndrome/EIEE</td>
<td>AD</td>
<td>1866</td>
<td>49</td>
<td>2.6%</td>
</tr>
<tr>
<td>TPP1</td>
<td>Neuronal ceroid lipofuscinosis</td>
<td>AR</td>
<td>1776</td>
<td>7</td>
<td>0.4%</td>
</tr>
<tr>
<td>SLC2A1</td>
<td>Glucose transporter deficiency</td>
<td>AD</td>
<td>1830</td>
<td>7</td>
<td>0.4%</td>
</tr>
<tr>
<td>ALDH7A1</td>
<td>Pyridoxine-dependent epilepsy</td>
<td>AR</td>
<td>1857</td>
<td>3</td>
<td>0.2%</td>
</tr>
<tr>
<td>SLC6A8</td>
<td>Cerebral creatine deficiency syndrome</td>
<td>XR</td>
<td>1795</td>
<td>2</td>
<td>0.1%</td>
</tr>
<tr>
<td>PNPO</td>
<td>Pyridoxamine 5'-phosphate oxidase deficiency</td>
<td>AR</td>
<td>1822</td>
<td>1</td>
<td>0.1%</td>
</tr>
</tbody>
</table>
Comparison to exome sequencing

- Mapping of epilepsy variants from three exome methods
  - Evaluated the sequencing coverage level of pathogenic epilepsy variants
    - Variants at low (<20x) coverage would likely be missed
  - ~1.6% of variants at low coverage
  - Comparable to recent publications [PMID:28152038]

- Meta-analysis of exome publications
  - Most variants in genes found on panels
    - For clearly defined epilepsy cases, 90% of finding are in epilepsy panels and another 5% are on other panels
  - Cost of panels is a small fraction of that of exomes
Conclusions

- Multi-gene panels have a high yield for epilepsy and cover most important genes at low cost and with fast turn-around time
  - 16-24% of patients had a positive molecular diagnosis (depending on panel)
  - 3 patients had dual diagnoses

- Important for NGS tests to include CNVs, large indels, etc.
  - In this cohort, 16.4% of positive findings were of these types

- Results with treatment implications are not a small proportion
  - 22% of molecular diagnoses in this cohort

- Some genetic causes of epilepsy are more common than anticipated
  - Pathogenic variants observed in PCDH19, SYNGAP1, PRRT2, DEPDC5

- Exome sequencing yields positive results in genes mostly found on panels and has technical limitations for hard-to-detect variants
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