Exciting Developments in the World of Epilepsy

Genetics

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With special thanks to Samuel F Berkovic, David Goldstein, Erin Heinzen, Heather Medford, Ruth Ottman, Elliott Sherr, Melodie Winawer, EPGP and Epi4K Investigators and Personnel

Disclosures

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- NINDS (EPGP, Epi4K, NETT)
- Epilepsy Study Consortium (unrestricted support from UCB, Lundbeck and the Finding a Cure for Epilepsy and Seizures Foundation)

To Cover:

- The reality of living with epilepsy
- How important is genetics as a cause of epilepsy?
  - Genetic epidemiology
- A brief overview of epilepsy gene discovery to date
- The next wave: identifying the genetic causes of more common, non-acquired epilepsies
A typical day in clinic…

• 21yo man with severe developmental delay and medically intractable epilepsy since early childhood. Having daily “small” seizures and monthly “big” seizures despite being on 4 AEDs and placement of a vagus nerve stimulator…

• 16yo student s/p left temporal lobectomy 10 years ago, with an apparent “fainting spell” 6 weeks ago…

Epilepsy: Impact on the Patient

• Seizures, typically unpredictable
• Risk of injury and death
• Co-morbidities
• Driving restrictions
• Underemployment and unemployment
• Lack of independence
• Stigma, discrimination and other social impacts

What is the worst thing about having epilepsy?

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Fisher. Epilepsy and Behavior 1:9-S14, 2000
Traditional View of Epilepsy Causation

Unknown
Idiopathic
Congenital
Trauma
Stroke
Others

Rochester Study
Hauser et al 1975

Genetic Epidemiology

Res Nerv Ment Dis 26:11, 1947

Figure: Photograph and EEG of Con-
sterned and kel, monstruous twins con-
sequent for cerebral abscess epi-
tomes, both with seizures onset at age 6
years. William Lennox, who both the
twine in 1945 at age 21 years.

Vadlamudi et al. Neurology 62:1127, 2004

Res Nerv Ment Dis 26:11, 1947

CHAPTER II
SIXTY-SIX TWIN PAIRS AFFECTED BY SEIZURES
WILLIAM G. LENNOX, M.D.

GENETICISTS are agreed that the study of twins provides the best method of evaluating the relative influence of genetic and acquired factors in the causation of human disease conditions. Monozygotic or single egg co-twins, have the same heredity, whereas the heredity of dizygotic co-twins is no greater than if they were siblings. In spite of the importance, this means of gaining information has been inadequately exploited. For the most part, reports have dealt with only one or a few twin pairs. A notable exception is the study of 1,000 monozygotic and 900 dizygotic twin pairs reported by Salemaa (1). No series of twins affected by seizures has been reported since the advent of electro-
encephalography. Because epilepsy is a paroxysmal cerebral dy-
rhythmia and because the brain wave pattern is an hereditary trait (2), the electroencephalogram can be used as a method of determining etiology and also as a means of studying the heredity of epilepsy.

Res Nerv Ment Dis 26:11, 1947

Fig. 3. The proportion of twin pairs in which both co-twins are or have been subject to
seizures. These twin pairs are divided into four groups, namely; monzygotic twins without
evidence of acquired brain pathology; and monzygotic twins with such evidence; third,
dizygotic twins without evidence of pathology; fourth, dizygotic twins with such evidence.
The solid portions represent those twin pairs in which both co-twins have chronic epilepsy; the
dotted portions indicate those in which one has chronic epilepsy, the other has had only
transient or febrile convulsions. The dotted area represents those twin pairs in which both
have had only transient or febrile convulsions.
### Genetic Epidemiology

**Twin Studies**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Monozygous (n)</th>
<th>Dizygous (n)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalised</td>
<td>0.73</td>
<td>0.33</td>
<td>0.0001</td>
</tr>
<tr>
<td>Focal</td>
<td>0.34</td>
<td>0.04</td>
<td>0.002</td>
</tr>
<tr>
<td>Febrile</td>
<td>0.60</td>
<td>0.14</td>
<td>0.0001</td>
</tr>
<tr>
<td>Unclassified</td>
<td>0.43</td>
<td>0.13</td>
<td>0.1</td>
</tr>
</tbody>
</table>

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**Case-wise concordance**

- Cohort study of descendants of parents of probands with epilepsy
- 196 cases of idiopathic epilepsy with seizure onset between 0 and 15 years, and 60 cases of isolated idiopathic seizures from Rochester, MN from 1935 and 1974

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### Risks of epilepsy in probands with epilepsy

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Observed Cases</th>
<th>Expected Cases</th>
<th>Relative Risk</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibs</td>
<td>12</td>
<td>4.8</td>
<td>2.5</td>
<td>1.3 - 4.4</td>
</tr>
<tr>
<td>Children</td>
<td>4</td>
<td>0.6</td>
<td>6.7</td>
<td>1.8 - 17.1</td>
</tr>
<tr>
<td>Nieces/nephews</td>
<td>3</td>
<td>2.4</td>
<td>1.3</td>
<td>0.3 - 3.7</td>
</tr>
<tr>
<td>Grandchildren</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-sibs</td>
<td>0</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grandnieces/nephews</td>
<td>0</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great-grandnieces/nephews</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Annegers et al. Neurology 32:174, 1982
To Cover:

- The reality of living with epilepsy
- How important is genetics as a cause of epilepsy?
  - Genetic epidemiology
- A brief overview of epilepsy gene discovery to date
- The next wave: identifying the genetic causes of more common, non-acquired epilepsies
**Epilepsy Genes: 2014**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARX</td>
<td>Infantile spasm, Early infantile epileptic encephalopathy</td>
</tr>
<tr>
<td>ATP1A2</td>
<td>Benign familial neonatal convulsions, Familial hemiplegic migraine and epilepsy</td>
</tr>
<tr>
<td>CACNA1A</td>
<td>Absence epilepsy and epileptic encephalopathy</td>
</tr>
<tr>
<td>CACNA1D</td>
<td>Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>CCR5 (ST80)</td>
<td>Infantile spasm</td>
</tr>
<tr>
<td>CHRNB4</td>
<td>Autosomal dominant nocturnal frontal lobe epilepsy, Infantile spasm</td>
</tr>
<tr>
<td>CHRNA2</td>
<td>Autosomal dominant nocturnal frontal lobe epilepsy</td>
</tr>
<tr>
<td>CHRNA4</td>
<td>Autosomal dominant nocturnal frontal lobe epilepsy, Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>CHRNA7</td>
<td>Absence epilepsy and episodic ataxia, Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>CACNA1A</td>
<td>Absence epilepsy and episodic ataxia, Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>CACNA1H</td>
<td>Juvenile myoclonic epilepsy, Early infantile epileptic encephalopathy</td>
</tr>
<tr>
<td>CACNB4</td>
<td>Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>CACNG1</td>
<td>Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>GABR1</td>
<td>Genetic epilepsy with febrile seizures plus</td>
</tr>
<tr>
<td>GABR2</td>
<td>Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>GABR3</td>
<td>Childhood absence epilepsy</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>Genetic epilepsy with febrile seizures plus, Benign familial neonatal convulsions, Ohtahara Syndrome</td>
</tr>
<tr>
<td>KCNQ4</td>
<td>Genetic epilepsy with febrile seizures plus, Benign familial neonatal convulsions, Malignant migrating partial epilepsy of infancy, Severe myoclonic epilepsy of infancy, Dravet Syndrome</td>
</tr>
<tr>
<td>KCNT1</td>
<td>Genetic epilepsy with febrile seizures plus, Severe myoclonic epilepsy of infancy, Dravet Syndrome</td>
</tr>
<tr>
<td>KCTD7</td>
<td>Progressive myoclonic epilepsy</td>
</tr>
<tr>
<td>LG1</td>
<td>Autosomal dominant familial epilepsy with auditory features</td>
</tr>
<tr>
<td>LG1A</td>
<td>Epilepsy in females with mental retardation</td>
</tr>
<tr>
<td>PLCA1</td>
<td>Early infantile epileptic encephalopathy</td>
</tr>
<tr>
<td>PRRT2</td>
<td>Infantile spasm, Early infantile epileptic encephalopathy, Partial onset epilepsy with intellectual disability</td>
</tr>
<tr>
<td>SCN1A</td>
<td>Genetic epilepsy with febrile seizures plus, Severe myoclonic epilepsy of infancy (Dravet syndrome)</td>
</tr>
<tr>
<td>SCN1B</td>
<td>Genetic epilepsy with febrile seizures plus</td>
</tr>
<tr>
<td>SCN2A</td>
<td>Benign familial neonatal convulsions, Genetic epilepsy with febrile seizures plus</td>
</tr>
<tr>
<td>SCN2B</td>
<td>Genetic epilepsy with febrile seizures plus</td>
</tr>
<tr>
<td>SCL2A1</td>
<td>Early-onset absence epilepsy, Epilepsy with paroxysmal exercise-induced dyskinesia</td>
</tr>
<tr>
<td>STXBP1</td>
<td>Early infantile spasm, Juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>TBC1D24</td>
<td>Familial neonatal convulsions, Paroxysmal tonic epilepsies of infancy, Early infantile epileptic encephalopathy</td>
</tr>
</tbody>
</table>

**The neurobiological spectrum of the epilepsies**

- **Genetic**
- **Structural/Metabolic**
- **Polygenic**
- **Idiopathic**
- **Congenital**
- **Unknown**
- **Trauma**
- **Stroke**
- **Others**

**Genetic/Epigenetic**

- **Epilepsies with polygenic inheritance**
- **Epilepsies with major acquired cause: trauma, infections, vascular etc.**

Modified from Helbig et al 2008

**To Cover:**

- The reality of living with epilepsy
- How important is genetics as a cause of epilepsy?
  - Genetic epidemiology
- A brief overview of epilepsy gene discovery to date
- The next wave: identifying the genetic causes of more common, non-acquired epilepsies
An international, multi-center, collaborative research effort funded by the National Institute of Neurological Disorders and Stroke designed to advance our understanding of the genetic basis of epilepsy

Ruben Kuzniecki, MD

The genesis of the project:
- First-hand awareness of the tremendous impact that epilepsy has on individuals and society, and the current limitations of what we offer our patients
- Recognition of accelerating advances in molecular analyses, and the pivotal role of phenomics
- Indisputable need for a national effort to achieve success
- Long-term impact of creating a national resource
- Enthusiasm for working with extremely motivated, talented, and willing collaborators

Overview of Study and Protocol

Overall Objective:
To create a database containing in-depth phenotype and genotype data from a large number of patients with epilepsy from throughout the United States, and to investigate the genetic influences on common and rare forms of epilepsy and pharmacoresistance.
Identify Potential Proband: Patient with IGE or LRE with a Full-Sibling with Nonacquired Epilepsy

Confirm Eligibility for Proband and Permission to Contact Sibling
  • Pre-screen
  • Consent
  • Screening Interview
  • Review of Medical Record, EEG, MRI

Blood Draw and Shipment to Coriell: Proband

Phenotyping of Proband and Sibling:
  • Diagnostic Interview
  • Medical Record Abstraction
  • Supplemental Forms
  • AED Data Sheet

Blood Draw and Shipment to Coriell: Sibling

Phenotyping Complete

Identify Potential Sibling: Full-Sibling of Proband with Nonacquired Epilepsy

Confirm Eligibility for Sibling
  • Pre-screen
  • Consent
  • Screening Interview
  • Review of Medical Record, EEG, MRI

Flow of Data for IGE/LRE Sib Pairs

<table>
<thead>
<tr>
<th>Study-Wide Enrollment as of 2/22/2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Enrollment</td>
</tr>
<tr>
<td>End of Month</td>
</tr>
<tr>
<td>Target</td>
</tr>
<tr>
<td>5,534</td>
</tr>
<tr>
<td>Consented</td>
</tr>
<tr>
<td>4,185</td>
</tr>
<tr>
<td>Eligible</td>
</tr>
<tr>
<td>3,750</td>
</tr>
<tr>
<td>1,500</td>
</tr>
</tbody>
</table>

33,816 activities 6,960,307 data points

Familial Aggregation of Seizure Semiology in the Epilepsy Phenome/Genome Project

Melodie Winawer, Robyn Fahlstrom, Catharine Shain, Daniel Rabinowitz and the EPGP Investigators

- Study of 1055 participants, including 412 with non-acquired focal epilepsy and 643 with generalized epilepsy.
- Familial aggregation was assessed by logistic regression analysis of relatives’ traits (dependent variable) by probands’ traits (independent variable), estimating the odds ratio for each symptom in a relative given presence versus absence of the symptom in the proband.

Focal Epilepsy
- Simple focal
- Complex focal
- Secondarily generalized tonic-clonic

Ictal Symptoms
- Motor
- Autonomic
- Psychic
- Aphasic

Generalized Epilepsy
- generalized tonic-clonic
- absence
- myoclonic
- atypical absence
- atonic
- reflex generalized

- Study-Wide Enrollment as of 2/22/2014
- Flow of Data for IGE/LRE Sib Pairs
- Familial Aggregation of Seizure Semiology in the Epilepsy Phenome/Genome Project
- 33,816 activities 6,960,307 data points
**Epi4K Project 1: Epileptic Encephalopathies (EE)**

- **Infantile Spasms (IS)**
  - 1 in 3000 live births and onset between 4-12 months of life
  - Characteristic chaotic interictal & EEG pattern of hypsarrhythmia, the *sine qua non* of the syndrome
  - 50-60% of IS cases have developmental brain malformations, tuberous sclerosis complex, chromosomal syndromes and metabolic conditions
  - Patients may evolve into LGS

- **Lennox-Gastaut syndrome (LGS)**
  - Onset between 1-8 years
  - Characterized by mixed seizure types and intellectual disabilities
  - Cause unknown in about 25-35% cases, symptomatic of structural or metabolic abnormalities

**EPGP: Epileptic Encephalopathies**

- Most EE do not show trans-generational transmission
- Most families do not have sibling recurrence
- **Hypothesis:**
  - Many IS/LGS patients have de novo causative mutations
- **Target families to highlight increased chance:**
  - Both biological parents available
  - No epilepsy in parents
  - No familial recurrence
- **Target genes that function at the “core of EE”**
  - Exclude patients with known causes
  - Exclude patients with severe Developmental Delay prior to seizure onset

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*De novo mutations in epileptic encephalopathies*

*Epileptic encephalopathies are a devastating group of severe childhood epilepsy disorders for which a cause is often unknown*. Since we report a screen for de novo mutations in patients with non-epileptic encephalopathies (EE): infantile spasms (IS), Lennox-Gastaut syndrome (LGS), and syndromic encephalopathies, we analyzed 204 probands and their parents, and confirmed IS in some cases. A whole-genome analysis showed a significant excess of de novo mutations in the IS/LGS genes that are the most likely to function at the brain’s interface, the IS/LGS network, and the EE-related brain phenotypes. We also find that those de novo mutations in two probands with genetic epilepsy are associated with epilepsy encephalopathy. Given the relative size of the IS/LGS network, the probability of these mutations occurring by chance are P = 4.2 × 10^-11 and P = 7.3 × 10^-6, respectively. Other genes with de novo mutations in this study include CC2D1A, CC2D2A, DNM1L, DNM1, CDK5RAP3, CNTN2, CDKL5, CDKL5, LGI1, CACNA1A, EPM2A, EPM2B, and ALG6/PRDH103. Finally, we show that the de novo mutations identified are enriched in specific gene sets including genes involved in the fragile X protein (P = 5.9 x 10^-4), as has been reported previously for autism spectrum disorders.

*were found to be highly penetrant (Table 1 and Fig. 1). We performed the same calculations on de novo mutations observed in children who had been treated with a specific regimen of drugs, valproic acid (VPA). Although results in this study are consistent with the de novo hypothesis, it is not clear whether this hypothesis is applicable to autism spectrum disorder*.

*In one common hypothesis, autism spectrum disorders (ASDs) are caused by de novo mutations that occur during meiosis or early embryogenesis, leading to an increase in mutation rates in these individuals. This hypothesis suggests that de novo mutations may play a role in the pathogenesis of ASDs.*

*In the future, high-throughput sequencing might allow for the identification of de novo mutations that are causative of autism spectrum disorders (ASDs).*

*Nature 501:217-221, 2013*
Distribution of *de novo* mutations

Number of *de novo* mutations per EE proband

Degree to which genes have more, or less, common functional variation than expected given the amount of presumably neutral variation they carry

Distribution of *de novo* mutations in intolerant genes

Genes with greater than one de novo SNV in 27 trios, and the probabilities of getting greater than or equal observed de novo mutation tally by chance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>Average effectively captured length (bp)</th>
<th>Weighted mutation rate</th>
<th>De novo mutation number</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>SCN1A</td>
<td>2</td>
<td>6064</td>
<td>1.6x10^-4</td>
<td>5</td>
<td>1.12x10^-9</td>
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<tr>
<td>STXBP1</td>
<td>9</td>
<td>1918</td>
<td>6.4x10^-5</td>
<td>5</td>
<td>1.16x10^-11</td>
</tr>
<tr>
<td>GABRB3</td>
<td>15</td>
<td>1207</td>
<td>3.78x10^-6</td>
<td>4</td>
<td>4.11x10^-16</td>
</tr>
<tr>
<td>CDKL5</td>
<td>X</td>
<td>2798</td>
<td>5.4x10^-5</td>
<td>3</td>
<td>4.90x10^-7</td>
</tr>
<tr>
<td>ALG13</td>
<td>X</td>
<td>475</td>
<td>1.03x10^-5</td>
<td>2</td>
<td>7.77x10^-10</td>
</tr>
<tr>
<td>DNM1</td>
<td>9</td>
<td>2323</td>
<td>9.1x10^-6</td>
<td>2</td>
<td>2.84x10^-4</td>
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<td>HDAC4</td>
<td>2</td>
<td>2650</td>
<td>1.16x10^-4</td>
<td>2</td>
<td>4.57x10^-4</td>
</tr>
<tr>
<td>SCN2A</td>
<td>2</td>
<td>5831</td>
<td>1.52x10^-4</td>
<td>2</td>
<td>1.14x10^-9</td>
</tr>
<tr>
<td>SCN8A</td>
<td>12</td>
<td>5814</td>
<td>1.64x10^-4</td>
<td>2</td>
<td>9.14x10^-4</td>
</tr>
</tbody>
</table>


12 likely de novo mutations in intolerant genes that are already disease-causing:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACNA1A</td>
<td>Episodic ataxia, familial hemiplegic migraine, ASD</td>
</tr>
<tr>
<td>CHD2</td>
<td>ASD and ID with seizures</td>
</tr>
<tr>
<td>FLNA</td>
<td>Periventricular heterotopia</td>
</tr>
<tr>
<td>GRN1</td>
<td>ID</td>
</tr>
<tr>
<td>GABRA1</td>
<td>ASD</td>
</tr>
<tr>
<td>GRN2B</td>
<td>Variety of neurodevelopmental phenotypes</td>
</tr>
<tr>
<td>HNRNPU</td>
<td>ID with seizures</td>
</tr>
<tr>
<td>IQSEC2</td>
<td>ID: one patient with infantile spasms</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>ID with seizures</td>
</tr>
<tr>
<td>KCNT1</td>
<td>ADNFLE and epilepsy of infancy with migrating focal seizures</td>
</tr>
<tr>
<td>MTOR</td>
<td>Hemimegalencephaly</td>
</tr>
<tr>
<td>NEDD4L</td>
<td>Photosensitive epilepsy and indirectly to infantile epilepsy</td>
</tr>
</tbody>
</table>

The de novo mutations are drawn preferentially from particular gene sets:
- Ion channels (p=1.3x10^-3)*
- Monogenic disorders with epilepsy (p=1.5x10^-2)
- ASD (p=9.4x10^-2)
- ID (p=7.8x10^-3)
- FMRP-regulated genes (p=4.2x10^-4)

* - after excluding genes known to cause EE

Clinical implications

- Significant genetic heterogeneity underlying IS and LGS
- These are the first mutations identified to be likely causative in LGS
- 3 mutations found in genes (MTOR, DCX, FNLA) associated with brain malformations but normal MRIs in all 3 patients
- 2 genes (SCN8A and GABRB3) each with de novo mutations in one pt. with IS and one pt. with LGS, despite no hx of IS in the LGS pt.
- 5 pts. with LGS with de novo mutations in SCN1A; in all 5 cases a re-review suggests these individuals had a clinical course consistent with Dravet syndrome despite an initial diagnosis of LGS
Conclusions

- The degree of suffering associated with epilepsy is horrible.
- We appear to again be on the upswing in deciphering the genetic architecture of the epilepsies.
- Large-scale, collaborative efforts are essential to future progress.
- The results are already having an important impact on clinical care.
- The latest EPGP/Epi4K results suggest that even though patients may carry very rare or private mutations conferring risk, many of the genes affected by these mutations can be organized into functionally-related groups. This may provide insight into the development of new therapies and individual treatment responses.

991 person-years of graduate-level training!

Thank you!