Clinical Genetic Diagnosis in Epileptic Encephalopathies

Heather Mefford, MD, PhD
University of Washington
December 9, 2013
Disclosure

NONE
Learning Objectives

• Review copy number variant testing in epilepsy

• Compare traditional and next-generation sequencing technologies

• Discuss the advantages and disadvantages of gene panels, whole exome and whole genome sequencing for clinical diagnosis
Genetic diagnosis & clinical care

• Knowing the genetic diagnosis....
  • Improves prognosis counseling
  • Facilitates discussion of recurrence risk
  • Affects medical management in some cases

• But...we only have a diagnosis in a fraction of cases
CNV testing in epilepsy

Recurrent CNVs

- 15q13, 16p13, 15q11
- Especially important in GGE
- Shared risk for autism, schizophrenia, ID

Non-recurrent CNVs

- Important in all types of epilepsy
- May involve known epilepsy genes
- Point to novel candidate genes
Who needs CNV testing?

- DD, ID, ASD, MCA
- ~12% (5-30%) yield
- 8% with rare CNV
- 4% clearly pathogenic

~3% of GGE

~10% of GGE + ID

Mullen et al., 2013, Neurology
Who needs CNV testing?

- Epileptic encephalopathy
- “Epilepsy +”
  - + brain malformation
  - + ID, ASD
  - + anomalies

4-10% diagnostic yield
# What is pathogenic?

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>Probably pathogenic?</th>
<th>Probably benign?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INHERITANCE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De novo</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Inherited – affected parent</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Inherited – healthy parent</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>PRESENT IN DATABASES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seen in control (unaffected)</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Database of Genomic Variants – projects.tcag.ca/variation/DECIPHER – decipher.sanger.ac.uk/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously reported in similarly affected patients</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>GENE CONTENT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNV contains morbid OMIM genes</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CNV is gene-rich</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CNV is gene-poor</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>CNV contains no regulatory elements</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Next-generation sequencing
Sequence analysis – the past 30 years

- Sanger sequencing
- PCR fragments > sequence > analyze
- Robust but low throughput
- First human genome: 10 years, $3 billion
Sequence analysis – the past 30 years

- Sanger sequencing
- PCR fragments > sequence > analyze
- Robust but low throughput
- First human genome: 10 years, $3 billion

Single gene analysis is typically performed using Sanger sequencing, e.g. SCN1A sequencing for Dravet syndrome
“Next-generation” sequencing

• Massively parallel sequencing

• Sequence millions of fragments simultaneously

• Your genome: a few weeks, ~$10,000
Gene panels for epilepsy diagnosis

35 genes
12/53 patients with mutation

- **SCN1A** (n=3)
- **SCN2A** (n=1)
- **STXBP1** (n=4)
- **CASK** (n=1)
- **CDKL5** (n=1)
- **MECP2** (n=1)
- microdel (n=1)

265 genes
16/33 patients with mutation

- **SCN1A** (n=6)
- **SCN2A** (n=1)
- **KCNJ10** (n=1)
- **KCNQ3** (n=1)
- **KCTD7** (n=1)
- **ARHGEF9** (n=1)
- **SMS** (n=1)
- **TPP1** (n=1)
- **MFSDE8** (n=1)
- **FLNA** (n=1)
Gene panels for diagnosis & discovery

500 EE patients
+ 65 genes

52 patients solved

Novel genes for EE described
Genetic heterogeneity in EE

101 genes
622 patients
72 (12%) solved

![Graph showing genetic heterogeneity in EE with specific genes and patient numbers.](image-url)
Clinical Gene Panels

- For clinical purposes, focus on known disease genes

$4500

- Glycosylation Disorders (23 genes)
- Early Infantile Encephalopathy (16 genes)
- Idiopathic Generalized Epilepsy (28 genes)
- Mitochondrial Dysfunction (27 genes)
- Inherited Metabolic Diseases (41 genes)
- Neurodegeneration (32 genes)

$4000-6000

- Joubert Syndrome (9 genes)
- Epilepsy in X-linked ID (18 genes)
- Brain or CNS Malformations (50 genes)
- Syndromic Disorders (71 genes)
- Other (12 genes)

$5000
Clinical Gene Panels

• Limited “space” to detect variants

• Any variants are found are in genes known to be important for the phenotype

• Parental testing may be necessary to interpret variants

• But….you don’t find what you’re not looking for
## What is pathogenic?

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>Probably pathogenic?</th>
<th>Probably benign?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INHERITANCE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>De novo</em></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Inherited – affected parent</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Inherited – healthy parent</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td><strong>PRESENT IN DATABASES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seen in control (unaffected)</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>individuals</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Exome Variant Server (6500 controls)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em><a href="http://snp.gs.washington.edu/EVS/">http://snp.gs.washington.edu/EVS/</a></em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously reported in similarly</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>affected patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AFFECT ON PROTEIN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes a conserved amino acid</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Creates a premature STOP codon</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Changes SPLICING</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Changes results of a FUNCTIONAL</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>assay</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Exome sequencing

“Capture” the exons of all ~20,000 genes

~300,000 exons
1% of the genome

~20,000 variants per individual

ACTTGCTAAGC

ACTTGC

C

CAGATG

Filter, analyze

ACTTGCTAAGC

TTGAAC
Gene discovery strategies

**Trio analysis**
- Sequence mom + dad + child
- Look for *de novo* change in child
- Severe, *de novo* disorders

**Family analysis**
- Sequence selected individuals
- Use inheritance pattern
- Recessive, dominant, X-linked

**Multiple unrelated affected**
- Sequence multiple individuals
- Look for mutations in the same gene across multiple affected

Project 1

Project 2
Exome sequencing: success stories

De Novo Pathogenic SCN8A Mutation Identified by Whole-Genome Sequencing of a Family Quartet Affected by Infantile Epileptic Encephalopathy and SUDEP

Krishna R. Veeramah,1 Janelle E. O'Brien,2 Miriam H. Meisler,1 Xiaoyang Sulayman D. Dib-Hajj,3 Stephen G. Waxman,5 Dinesh Talwar,5,6 Santhi Evan E. Eichler,5,6 Linda L. Restivo,2,3,6 Robert P. Erickson,3,6 and Michael DePape,1

Mutations in TNK2 in Severe Autosomal Recessive Infantile Onset Epilepsy

Yuki Hitomi PhD,1 Erin L. Heinzen PhD,1 Simona Donatello PhD,2 et al.

Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome

Sarah B Ng1,2, Abigail W Bigham2,3, Kati J Buckingham2, Mark C Hamel2, Heidi I Gildersleeve3, Anita E Beck3, Holly K Tabor2,3, Gregory M Cox1,2,3, Emily H Turner1, Joshua D Smith1, Mark J Rieder4, Koh-ichiro Yoshida5, Norio Niikawa6, Deborah A Nickerson1, Michael J Bamshad1,2 & Jay S. Appleby1

De novo mutations of SETBP1 cause Schinzel-Giedion syndrome

Alexander Hoischen2,4, Breggie W M van Bon1,4,1, Christian Gilissen1,4, Peer Arts1, Bart van Lier1, Marloes Steenhour1, Petra de Vries1, Rick de Reus1, Ineke Wieskamp1, Geert Mortier2, Koen Devriendt1, Marta Z Amorim2, Nicole Revenu2, Alexa Kidd4, Mafalda Barbosa6, Anne Turner6, Janine Smith6, Christina Oley10, Alex Henderson11, Ian M Hayes12, Elizabeth M Thompson13, Han G Brunner1, Bert B A de Vries4 & Joris A Veltman3

Diagnostic Exome Sequencing in Persons with Severe Intellectual Disability

Joep de Ligt, M.Sc.1, Marjolein H. Willemsen, M.D., Breggie W.M. van Bon, M.D., Ph.D.3, Tijtske K. Hoogerbrugge1,2, Annemiek Pettenkofer1, Petra de Vries1, Alexander

De novo mutations in epileptic encephalopathies

Epi4K Consortium* & Epilepsy Phenome/Genome Project*
Epi4K: Exome sequencing

264 trios with Infantile Spasms or Lennox Gastaut syndrome

*SCN1A, SCN2A, SCN8A, STXBP1, CDKL5*

*GABRB3* – 4 patients with de novo mutations

*ALG13* – 2 patients with identical mutations

*HDAC4, DNM1* – 2 patients each

Sequence mom + dad + child

Look for *de novo* change in child

Severe, *de novo* disorders
Exome sequencing for diagnostics

- Trio exome sequencing in 100 patients with severe ID (IQ<50)
- For 3 genes – sequenced 765 additional cases to find a second mutation
- 16% diagnostic yield

Validation studies in large cohorts may be required for new genes
May not be feasible for every gene in a clinical setting
Clinical exome sequencing

250 probands referred for WES

80% children with “neurological phenotype”

62 patients (25%) with likely diagnosis
  33 AD (83% de novo)
  16 AR
  9 XL (40% de novo)

4 probands with 2 diagnoses
Clinical exome sequencing

• Selection of patients / families is important

• Additional family members are helpful

• Interpretation is guided by family history, disease information

• Cost ~$5,000-10,000 and falling...

• Turnaround time 8-16 weeks
Advantages

• Expanded genomic “space” in which to detect variants

• Phenotypic spectrum for some disorders/genes is expanding

• Single test rather than multiple, serial tests
Cautions

• You might find what you’re not looking for...

• ACMG guidelines – “incidental” findings

• Interpretation
What is reported?
What about my patient?

Suspect a specific diagnosis?
- Dravet: SCN1A
- Rett: MECP2

Nonspecific, epilepsy+

No answers
- Interesting phenotype
- Interesting family (Good insurance)

Many possible causative genes

CNV testing

Whole exome

Gene panel
Impact on Clinical Care and Practice

• Diagnosis for the patient and their family

• Improved prognosis counseling

• Recurrent risk counseling

• Further research to understand appropriate and effective therapies
Acknowledgments

**Mefford Lab**
Gemma Carvill
Joe Cook
Adiba Khan
Matty Zemel
Steffie Beijnsberger
Candace Myers
Eileen Geraghty
Corinna Hartmann

**UW**
Jay Shendure
Brian O’Roak
Evan Eichler
Michael Dorschner

**U Melbourne**
Ingrid Scheffer
Sam Berkovic
Jacinta McMahon
Sinead Heavin

**Epi4K**
David Goldstein
Sam Berkovic
Dan Lowenstein
Elliott Sherr
Ingrid Scheffer
Evan Eichler
Ann Poduri
Dennis Dlugos