Clinical Exome Analysis

Diana Kolbe, Ph.D.
9 June 2016
Introduction

• My work: bioinformatic analysis, research and clinical
• Clinical exome test
  • Requested by UIHC physician
  • CLIA-certified, CAP-accredited medical testing
  • Results returned – electronic health record
• Not just me!
  • Physician(s)
  • Genetic counselor
  • Lab personnel and sequencing core
  • Additional subject area experts as needed
What differentiates a *clinical* exome?

- Standardized analysis procedure
- QC/confidence requirements
- Reporting
  - No gene discovery
  - ACMG guidelines on variant classification
  - Must validate all findings by additional method
  - Consent on incidental and secondary findings
- Physician education
  - Limitations of an exome study
  - What does a negative result mean?
- Genetic counseling for family, pre- and post-test
WES test workflow

Healthcare provider (HCP) identifies patient/family

Patient and family members agree to test

Mycah and Colleen will work with HCP and patient to schedule genetic counseling appts. or to refer to a CGC upon request

Patient meets with IIHG Genetic Counselor & signs consent form (notify Mycah consent was obtained)

HCP completes completes & submits requirements found on IIHG website: Phenotips, Requisition, Pedigree, Family History, signed consent forms

IIHG website: Education modules available online

Mycah receives information and samples. Mycah checks we have all required information. Creates a paper and an electronic folder on R drive.

Wednesday Clinical Team Meeting:
(Mycah to take minutes and distributes to team following meeting)
1. Analyzes the pedigree to determine the study design
2. Determines Bioinformatics analysis plan including a candidate gene list from GeneTests, Phenotips
3. Plan stored in folder
4. Mycah complete 1st section of final report

Colleen generates a preliminary candidate gene list from:
1. HCP suggested genes
2. GeneTests (based on Phenotips input)

Upon receipt of all materials, Mycah will notify the group that we will be meeting for the CDS Meeting (Wednesday’s at noon)
Diana will be invited to the meeting

Mycah notifies HCP if any information is incomplete

Colleen reviews all materials and notifies Mycah if information is incomplete.

Carla emails Jen & Einat number of samples for IIHG Clinical Exome que

Colleen updates candidate gene list after clinical team meeting review and saves it in the IIHG R drive for Bioinformatics team use

Sara performs DNA extraction Brings samples to Jen/Einat

Genomics Division (Jen/Einat) performs sequencing on HiSeq 2000 (eventually HiSeq2500) and notifies Diana/Tom/IIHG when complete

Diana, Tom, IIHG downloads data

IIHG Bioinformatics Division (Diana) performs alignment, variant calling, genomic annotation, and coverage analysis
Filter variants to candidate gene list
Diana sends Adela & Colleen the excel variant list and coverage tables

Adela validates final variant list by Sanger Sequencing
Reports results to CDD team.

Adela/Colleen perform filtering based on candidate gene list

Mycah contacts HCP to schedule a meeting to review results

Optional: IIHG (Colleen, Adela, Diana) meet to discuss preliminary results

Mycah and Colleen will work with HCP and patient to schedule genetic counseling appts. or to refer to a CGC upon request

IHG Genetic Counselor assists the HCP in presenting results to patient and family & providing genetic counseling
OR
HCP provides genetic counseling

Optional IIHG-HCP Consultation & Support Service
Pager #1576 OR phone 319-335-3688

HCP follow-up survey?
IIHG evaluates the findings for possibility of diagnostic test development
Manuscripts

Diana/Tom/IIHG stores the following data in the ________ location for one year:
1. Fastq on IIHG Storage drive
2. Filtered and annotated variant list on IIHG R drive

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Physician orders test

- What makes a good clinical case?
  - Some diseases have better solve rates
  - Recessive/sporadic cases are more successful than dominant with small families
  - Nuclear trio always recommended
  - Thorough case history

- Competing with GeneDx
  - We are not the cheapest, or the fastest
  - Advantages to collaborative approach
<table>
<thead>
<tr>
<th>Family ID</th>
<th>Disease presentation</th>
<th>Sequenced</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIHG-112</td>
<td>Severe epilepsy</td>
<td>Quad</td>
<td><em>PIGA</em> (X-linked recessive)</td>
</tr>
<tr>
<td>IIHG-116</td>
<td>Severe epilepsy</td>
<td>Trio</td>
<td><em>KARS</em> (recessive)</td>
</tr>
<tr>
<td>IIHG-117</td>
<td>Severe epilepsy</td>
<td>Trio</td>
<td><em>DNM1</em> <em>(de novo)</em></td>
</tr>
<tr>
<td>IIHG-129</td>
<td>Severe epilepsy</td>
<td>Trio</td>
<td><em>KCNT1</em> <em>(de novo)</em></td>
</tr>
<tr>
<td>IIHG-130</td>
<td>Severe epilepsy</td>
<td>Trio</td>
<td>negative</td>
</tr>
<tr>
<td>IIHG-136</td>
<td>Muscular dystrophy (dominant)</td>
<td>Trio</td>
<td>negative</td>
</tr>
<tr>
<td>IIHG-151</td>
<td>Multiple congenital anomalies</td>
<td>Trio</td>
<td>negative (identified variants may contribute, but not fully explanatory)</td>
</tr>
<tr>
<td>IIHG-158</td>
<td>Severe epilepsy</td>
<td>Trio</td>
<td><em>DNM1</em> <em>(de novo)</em></td>
</tr>
<tr>
<td>IIHG-159</td>
<td>Severe epilepsy</td>
<td>Trio</td>
<td>negative</td>
</tr>
<tr>
<td>IIHG-163</td>
<td>Muscular dystrophy/Charcot-Marie-Tooth</td>
<td>Singleton</td>
<td>negative</td>
</tr>
</tbody>
</table>
Pre-test genetic counseling

- Important in both directions!

- We get
  - detailed pedigree information
  - separate consents for: primary results, incidental findings, database participation, etc.
  - particular areas of concern

- Family gets
  - Guidance on what test can and can’t provide (expectations)
  - Control over what they learn and how information is used
Pre-analysis

- Sample collection
- Payment approval: Insurance? Medicaid? Out of pocket?
- DNA extraction
- Exome library creation
- Sequencing
Analysis plan

- Conducted before sequencing data is returned
- Phenotype and pedigree review
- Inheritance modes to consider (with priority)
- Candidate gene list
  - Previous genetic testing in patient
  - Gene panels for disease
  - Known genes for phenotype(s)
  - Face2Gene
  - etc.
QC analysis

**Total Reads**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIHG-116-2</td>
<td>120000000</td>
</tr>
<tr>
<td>IIHG-116-3</td>
<td>100000000</td>
</tr>
<tr>
<td>IIHG-116</td>
<td>140000000</td>
</tr>
</tbody>
</table>

**% of Reads Mapped**

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Mapped</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIHG-116-2</td>
<td>0.92</td>
</tr>
<tr>
<td>IIHG-116-3</td>
<td>0.9</td>
</tr>
<tr>
<td>IIHG-116</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**frac. Target Covered 30X**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIHG-116-2</td>
<td>0.96</td>
</tr>
<tr>
<td>IIHG-116-3</td>
<td>0.94</td>
</tr>
<tr>
<td>IIHG-116</td>
<td>0.96</td>
</tr>
</tbody>
</table>

**frac. Reads Overlapping Target**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Overlap</th>
</tr>
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<tbody>
<tr>
<td>IIHG-116-2</td>
<td>0.7</td>
</tr>
<tr>
<td>IIHG-116-3</td>
<td>0.6</td>
</tr>
<tr>
<td>IIHG-116</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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*Note: The graphs and tables above represent the quality control analysis of RNA sequencing data for three samples: IIHG-116-2, IIHG-116-3, and IIHG-116.*
Data Quality

• Excellent
  • >99.5% covered at 10x or better
  • IIHG-116: 97.2% covered at 30x or better
  • IIHG-116-2: 97.5% covered at 30x or better
  • IIHG-116-3: 96.9% covered at 30x or better
Filtering

- Variant-level quality check
  - minimum QUAL value
  - minimum depth
  - minimum QUAL/depth (QD)
- % observed
  - Ideally, a variant should be seen in in ~50% or ~100% of reads
  - SNVs seen at <20% are unlikely to be real variants
  - Indels tend to be under-represented (worse for larger)
  - A 20-base deletion observed at 15% might be real – missing 35% are mis-aligned giving slightly different variants
- Rarity: a common variant should not cause a rare disease
Inheritance mode filtering

- **de novo**
  - het in child
  - evidence of absence in parents
  - very rare

- **dominant**
  - het in child
  - het in affected parent
  - absent in unaffected parent
  - very rare

- **homozygous recessive**
  - homozygous in child
  - het in each parent

- **compound heterozygous recessive**
  - more than one het variant in child
  - at least one inherited from each parent

- **X-linked recessive**
  - male child
  - homozygous on chrX
  - het in mother
  - absent in father

- **known pathogenic**
  - reported in ClinVar as pathogenic
  - OR reported in OMIM, HGMD…
  - reported in ClinVar as pathogenic
  - OR reported in OMIM, HGMD…
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  - OR reported in OMIM, HGMD…
  - reported inClinVar as pathogenic
  - OR reported in OMIM, HGMD…
  - reported inClinVar as pathogenic
Prioritization

- Ontologies!
- Human Phenotype Ontology: how much overlap is there between my patient’s phenotype and the terms associated with a gene with a variant
- More complicated methods add (e.g.) gene-gene interactions, model system data
- For example: de novo variant in gene known to interact with a gene that has a very similar known phenotype
  - Not clinically reportable!
  - May help determine whether to move patient to a research study
Coverage analysis

- An exome aims to sequence all coding exons (non-coding depends on exome kit selected)
- No platform, no experiment is perfect
- Region of interest not included in exome kit? Did not sequence well in this sample?
- For genes of interest, are there regions that don’t have sufficient reads for variant calling?
Internal discussion

• Begin report write-up
• If positive
  • Plan validation experiments – usually Sanger sequencing
• If negative
  • Review variants of unknown significance
  • Discuss follow-up strategies or additional analyses
  • Are there recessive-model candidate genes with one variant and also poor coverage regions? Is additional testing warranted?
ACMG: Evidence framework

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<th>Benign</th>
<th>Supporting</th>
<th>Pathogenic</th>
</tr>
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<tbody>
<tr>
<td>MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2</td>
<td>Strong</td>
<td>Supporting</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prevalence in affecteds statistically increased over controls PS4</td>
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<th>Benign</th>
<th>Supporting</th>
<th>Pathogenic</th>
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<td>Multiple lines of computational evidence suggest no impact on gene /gene product BP4</td>
<td>Strong</td>
<td>Supporting</td>
<td>Strong</td>
</tr>
<tr>
<td>Missense in gene where only truncating cause disease BP1</td>
<td></td>
<td></td>
<td>Very strong</td>
</tr>
<tr>
<td>Silent variant with non predicted splice impact BP7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-frame indels in repeat w/out known function BP3</td>
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<tr>
<td>Well-established functional studies show no deleterious effect BS3</td>
<td>Strong</td>
<td>Supporting</td>
<td>Moderate</td>
</tr>
<tr>
<td>Missense in gene with low rate of benign missense variants and path. missenses common PP2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutational hot spot or well-studied functional domain without benign variation PM1</td>
<td></td>
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<td>Well-established functional studies show a deleterious effect PS3</td>
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<th>Benign</th>
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<tbody>
<tr>
<td>Nonsegregation with disease BS4</td>
<td>Strong</td>
<td>Supporting</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cosegregation with disease in multiple affected family members PP1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Increased segregation data</td>
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</thead>
<tbody>
<tr>
<td>De novo (without paternity &amp; maternity confirmed) PM6</td>
<td>Strong</td>
<td>Supporting</td>
<td>Very strong</td>
</tr>
<tr>
<td>De novo (paternity and maternity confirmed) PS2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allelic data</th>
<th>Benign</th>
<th>Supporting</th>
<th>Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed in trans with a dominant variant BP2</td>
<td>Strong</td>
<td>Supporting</td>
<td>Strong</td>
</tr>
<tr>
<td>Observed in cis with a pathogenic variant BP2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For recessive disorders, detected in trans with a pathogenic variant PM3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other database</th>
<th>Benign</th>
<th>Supporting</th>
<th>Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reputable source w/out shared data = benign BP6</td>
<td>Strong</td>
<td>Supporting</td>
<td>Moderate</td>
</tr>
<tr>
<td>Reputable source = pathogenic PP5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other data</th>
<th>Benign</th>
<th>Supporting</th>
<th>Pathogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found in case with an alternate cause BP5</td>
<td>Strong</td>
<td>Supporting</td>
<td>Moderate</td>
</tr>
<tr>
<td>Patient's phenotype or FH highly specific for gene PP4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ACMG Classification

### Table 5 Rules for combining criteria to classify sequence variants

<table>
<thead>
<tr>
<th>Classification</th>
<th>Rules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>(i) 1 Very strong (PVS1) AND &lt;br&gt; (a) ≥1 Strong (PS1–PS4) OR &lt;br&gt; (b) ≥2 Moderate (PM1–PM6) OR &lt;br&gt; (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) OR &lt;br&gt; (d) ≥2 Supporting (PP1–PP5) &lt;br&gt; (ii) ≥2 Strong (PS1–PS4) OR &lt;br&gt; (iii) 1 Strong (PS1–PS4) AND &lt;br&gt; (a) ≥3 Moderate (PM1–PM6) OR &lt;br&gt; (b) 2 Moderate (PM1–PM6) AND ≥2 Supporting (PP1–PP5) OR &lt;br&gt; (c) 1 Moderate (PM1–PM6) AND ≥4 supporting (PP1–PP5)</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>(i) 1 Very strong (PVS1) AND 1 moderate (PM1–PM6) OR &lt;br&gt; (ii) 1 Strong (PS1–PS4) AND 1–2 moderate (PM1–PM6) OR &lt;br&gt; (iii) 1 Strong (PS1–PS4) AND ≥2 supporting (PP1–PP5) OR &lt;br&gt; (iv) ≥3 Moderate (PM1–PM6) OR &lt;br&gt; (v) 2 Moderate (PM1–PM6) AND ≥2 supporting (PP1–PP5) OR &lt;br&gt; (vi) 1 Moderate (PM1–PM6) AND ≥4 supporting (PP1–PP5)</td>
</tr>
<tr>
<td>Benign</td>
<td>(i) 1 Stand-alone (BA1) OR &lt;br&gt; (ii) ≥2 Strong (BS1–BS4)</td>
</tr>
<tr>
<td>Likely benign</td>
<td>(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) OR &lt;br&gt; (ii) ≥2 Supporting (BP1–BP7)</td>
</tr>
<tr>
<td>Uncertain sign</td>
<td>(i) Other criteria shown above are not met OR &lt;br&gt; (ii) the criteria for benign and pathogenic are contradictory</td>
</tr>
</tbody>
</table>
Results meeting

• Includes physician, counselor, lab staff, bioinformatics, administrative, etc.
• Not just a yes/no answer – go over the whole process
• Education opportunity for physician, especially on first submissions
Post-test genetic counseling

- Return results to family
- Implications for patient – what are outcomes for patients with mutations in this gene? Does this change case management?
- Should additional family members be tested?
- Sociological questions: how much information will you share with friends and family? Does everyone agree?
- If negative – interested in research enrollment? For example, Epilepsy Genomics Initiative
Case example

- Phenotype: infant male with intractable epilepsy, microcephaly, nystagmus, and cortical visual impairment
- Parent-child trio with no siblings
- Some paternal relatives with epilepsy (less severe)
Variant filtering strategy

- Standard filters: variant quality, coding or splicing change, and minor allele frequency

- Many possible inheritance modes
  - De novo
  - Autosomal or X-linked recessive
  - Autosomal dominant, incomplete penetrance
  - Mitochondrial
## Results: recessive variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant location</th>
<th>HGVS</th>
<th>Zygosity</th>
<th>Exon/Intron</th>
<th>Variant Type</th>
<th>dbSNP_ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAIAP2L2</td>
<td>chr22:38483156-&gt;TCATGGGTG</td>
<td>NM_025045:c.1234_1235insCACCCATGA</td>
<td>homozygote_alternate</td>
<td>EXON11</td>
<td>in_frame_inser+on</td>
<td>rs142739979</td>
</tr>
<tr>
<td>DNAH6</td>
<td>chr2:85043186:G&gt;A</td>
<td>NM_001370:p.Ala4118Thr</td>
<td>homozygote_alternate</td>
<td>EXON76</td>
<td>non-synonymous</td>
<td>rs72836490</td>
</tr>
<tr>
<td>HEPH</td>
<td>chrX:65486284:G&gt;T</td>
<td>NM_001130860:c.3254-1G&gt;T</td>
<td>homozygote_alternate</td>
<td>INTRON20</td>
<td>canonical splice</td>
<td>rs35700738</td>
</tr>
<tr>
<td>HS6ST2</td>
<td>chrX:132092485:G&gt;A</td>
<td>NM_147175:p.Ser49Leu</td>
<td>homozygote_alternate</td>
<td>EXON2</td>
<td>non-synonymous</td>
<td>rs181526961</td>
</tr>
<tr>
<td>KARS</td>
<td>chr16:75675599:C&gt;G</td>
<td>NM_005548:p.Ala29Pro</td>
<td>homozygote_alternate</td>
<td>EXON2</td>
<td>non-synonymous</td>
<td></td>
</tr>
<tr>
<td>MYH7B</td>
<td>chr20:33586598:A&gt;C</td>
<td>NM_020884:p.Glu1399Ala</td>
<td>heterozygote</td>
<td>EXON35</td>
<td>non-synonymous</td>
<td>rs202122911</td>
</tr>
<tr>
<td>NAV1</td>
<td>chr1:201777202:A&gt;G</td>
<td>NM_020443:p.Glu1257Arg</td>
<td>heterozygote</td>
<td>EXON18</td>
<td>non-synonymous</td>
<td></td>
</tr>
<tr>
<td>OCRL</td>
<td>chrX:128674722:C&gt;T</td>
<td>NM_000276:p.Thr14Ile</td>
<td>homozygote_alternate</td>
<td>EXON2</td>
<td>non-synonymous</td>
<td>rs61752970</td>
</tr>
<tr>
<td>PLEKH1N</td>
<td>chr1:901922:G&gt;C</td>
<td>NM_001360184:p.Ser4Thr</td>
<td>heterozygote</td>
<td>EXON1</td>
<td>non-synonymous</td>
<td>rs62639980</td>
</tr>
<tr>
<td>RPGR</td>
<td>chrX:38164037:G&gt;C</td>
<td>NM_001034853:p.Ala262Gly</td>
<td>homozygote_alternate</td>
<td>EXON8</td>
<td>non-synonymous</td>
<td>rs138018739</td>
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<tr>
<td>ZNF81</td>
<td>chrX:47705674:C&gt;T</td>
<td>NM_007137:p.Ala3Val</td>
<td>homozygote_alternate</td>
<td>EXON2</td>
<td>non-synonymous</td>
<td>rs183846665</td>
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</tbody>
</table>
## Results: best recessive variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant location</th>
<th>HGVS</th>
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<th>Variant Type</th>
<th>dbSNP_ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>KARS</td>
<td>chr16:75675599:C&gt;G</td>
<td>NM_005548:p.Ala29Pro</td>
<td>homozygote_alternate</td>
<td>EXON2</td>
<td>non-synonymous</td>
<td></td>
</tr>
<tr>
<td>NAV1</td>
<td>chr1:201777202:A&gt;G</td>
<td>NM_020443:p.Gln1257Arg</td>
<td>heterozygote</td>
<td>EXON18</td>
<td>non-synonymous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>chr1:201617862:G&gt;C</td>
<td>NM_020443:p.Glu22Asp</td>
<td>heterozygote</td>
<td>EXON1</td>
<td>non-synonymous</td>
<td></td>
</tr>
</tbody>
</table>

**Best candidates:**
- **KARS novel variant**
  - Apparent non-Mendelian inheritance: father is het, mother is reference
  - Some evidence for a multi-exon deletion spanning this site in mother
  - Known phenotypes are Charcot-Marie-Tooth recessive intermediate B and non-syndromic deafness
- **NAV1 compound het novel variants**
  - Unknown gene function, similar to *C. elegans* gene unc-53 involved in axon guidance
  - EXON1 variant only included in longest transcript form
Results: possible *de novo* variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant location</th>
<th>HGVS</th>
<th>Zygosity</th>
<th>Exon/Intron</th>
<th>Variant Type</th>
<th>dbSNP_ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC</td>
<td>chr5:112824069:T&gt;C</td>
<td>NM_001085377:c.A43G:p.S15G</td>
<td>heterozygote</td>
<td>EXON1</td>
<td>non-synonymous</td>
<td>rs201571604</td>
</tr>
<tr>
<td>MN1</td>
<td>chr22:28194912:T&gt;C</td>
<td>NM_002430:c.A1620G:p.Q540Q</td>
<td>heterozygote</td>
<td>EXON1</td>
<td>synonymous</td>
<td>rs45520238</td>
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<tr>
<td>MN1</td>
<td>chr22:28194933:T&gt;C</td>
<td>NM_002430:c.A1599G:p.Q533Q</td>
<td>heterozygote</td>
<td>EXON1</td>
<td>synonymous</td>
<td></td>
</tr>
</tbody>
</table>

All appear to be the result of difficult-to-align low-complexity sequence, rather than true *de novo* variants.
Results: dominant variants

- Presumed incomplete penetrance from father’s side, which has some history of epilepsy
- 192 good quality rare variants present in father and child, but not mother
- Filtering for a dominant cause in a trio is very inefficient
Results: mitochondrial variants

- Child’s mtDNA identical to maternal
Our initial analysis: negative with interesting recessive variants

- Presented to team
- Physician reported back 1-2 wks later – found a paper newly reporting *KARS* for an early infantile epileptic encephalopathy phenotype – excellent case match
Reducing the Cost of the Diagnostic Odyssey in Early Onset Epileptic Encephalopathies

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