Clinical Whole Exome Sequencing: For the Evaluation of Genetic Disorders

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The patient with a suspected genetic disorder poses a challenge to diagnosis

- Thousands of genetic disorders
- Rare
  - Cystic fibrosis 1:2500
  - Hunter syndrome 1:150,000
- Clinical heterogeneity
- Specialized testing needed to confirm diagnosis
  - 3.5 geneticists per 1 million population
- Many patients do not have a diagnosis
Diagnostic Yield of Common Genetic Tests

Karyotype 5 - 15%

Array-CGH – 15-20%

Pickup Rate for Selected Sanger Tests at MGL
Prior Studies of Exome Approaches to Cohorts of Undiagnosed Patients

  – Selected patients for undiagnosed genetic disorder
  – Battery of diagnostic approaches
  – 24% diagnostic rate

  – Selected cohort of severe intellectual disability
  – Molecular diagnostic rate through exome of 16%
Whole Exome Sequencing:

CLINICAL UTILITY IN A CLINICAL DIAGNOSTIC LAB
Whole Exome Sequencing Workflow

Sample Intake

DNA Extraction, SNP-Array

Library Preparation

Target Enrichment

Illumina Sequencing

Data analysis

Clinical Report

QC steps at each stage

1) Mapping: <4% error rate, >90% unique reads
2) Data analysis: >10 Gb data, >95% target bases >20X, >85% target bases >40X, mean coverage >140X
3) SNP concordance to genotype array: >99.8%
Baylor WES: 97% of Target Covered at 20+ Times

12 Batches of Clinical Samples

<table>
<thead>
<tr>
<th>Total Gb/Sample</th>
<th>Unique Aligned Gb</th>
<th>Unique Reads</th>
<th>Coverage</th>
<th>On Target</th>
<th>Targets hit</th>
<th>Bases 20+ Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.48</td>
<td>12.73</td>
<td>95.80%</td>
<td>168</td>
<td>76%</td>
<td>99.40%</td>
<td>97%</td>
</tr>
</tbody>
</table>

Robust lab performance to deliver high-standard consistency.
Analysis Pipeline (Mercury v1.0)

- Data from Illumina Instrument
- bcl, .bam, .fastq
- CASAVA
- BWA
- Atlas-SNP
- Cassandra SNP
- MCP
- eGenoType concordance
- final.bam, snp.vcf, indel.vcf
- "final.vcf"
- QC
- LIMS

SNP array data
Variant Filtering for Clinical WES

VCF, 200k variants

Quality Filter?

In HGMD?

Change on Splicing or Protein

MAF < 1%

MAF <5% in public databases

MAF <2% in Baylor databases

CASSANDRA
Fine-tuned filtering for clinical samples.

Output File (400-700 Variants)
Variant Interpretation

- Correlation with patient phenotype
- Public databases
  - ESP (NHLBI GO Exome Sequencing Project), TG
  - OMIM, HGMD, GeneTests, LSDB
- Internal knowledge base from 800 Clinical Exomes
  - Curated lists of variant classifications
    - Internally annotated mutations/VUS lists
    - Common variants
  - New gene list updated by WGL weekly
Clinical Reporting of Whole Exome

• Sign out team of ABMG-certified laboratory directors, medical directors, clinicians, genetic counselors
• Three levels of review
  – Disease-gene association, functional prediction, in silico prediction
• Focused and expanded report
• Components of WES
  – Sequence result
  – Sanger confirmation, parental inheritance of significant findings
  – Mitochondrial genome
Disease Phenotype

• Detailed phenotype informs analysis
• Questionnaire by organ system
• Request clinic note
Focused Report: Based on Disease Phenotype

- Deleterious mutations in disease genes related to clinical phenotype
- Variants of unknown clinical significance in genes related to phenotype
- Immediately “medically actionable” mutations
  - Marfan, NF1, VHL, MEN2A
- Autosomal recessive carrier status
- Pharmacogenetic loci
Medically Actionable Definition

• Finding with direct clinical utility based on established guidelines and/or medical literature
• Availability of treatment or established guidelines for disease prevention
• Unrecognized secondary diagnosis: Marfan, NF1, NF2
• Preventable disease: HNPCC, BRCA1,2
Whole Exome Report: Expanded Report

- Deleterious mutations in genes apparently unrelated to phenotype
- Variants of unknown significance
  - For AR, a deleterious mutation in same gene must also be present
- Predicted clearly deleterious mutations in genes with no current association with disease
Baylor Experience with Clinical Exome Sequencing
BCM WGL Launches Whole Exome Sequencing Oct 2011

- ~1500 samples
- 85% peds; 15% adult
- Mostly neurologic
- In addition: skeletal disorders, pulmonary artery hypertension, cardiovascular dz
- Variety of referral sources – academic medical centers
Samples Referred by Specialty

Referral Specialty

- Genetics: 61%
- Neurology: 12%
- Pediatrics: 24%
- Other: 3%

Legend:
- Genetics
- Neurology
- Pediatrics
- Other
Of over 1,200 samples received since November 2011, 760 samples have been finalized

Causative deleterious mutations related to patient phenotype have been identified in a minimum of 25% (190) patients

- 52% (99) of the positive cases are AD disorders
- 33% (62) of the positive cases are AR disorders
- 12% (22) of the positive cases are X-linked disorders
- 4% (7) of the positive cases have two molecular disorders
## Molecular Diagnoses in Mendelian Disorders

**Positive Rate: 62/250, ~25%**

<table>
<thead>
<tr>
<th>Inherit.</th>
<th>Genes with mutant alleles (times observed)</th>
<th>de novo mutants (%)</th>
<th>Novel variants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AD</strong></td>
<td>ANKRD11 (2), <strong>ARID1B (2)</strong><em>, ATL1 (2), <strong>KRAS (2)</strong> ¶; ABCC9, ARID1A, <strong>CBL</strong>¶, CHD7, COL3A1, CREBBP, CRYGD, DYRK1A, EP300, FGFR1, <strong>HDAC8</strong>§, ITPR1, KANSL1, KAT6B, KIF1A, MLL2, <strong>NIPBL</strong>§, PTEN, <strong>PTPN11</strong>¶, SCN2A, SCN8A, SETBP1, SHANK3, <strong>SMARCB1</strong></em>, SPAST, SRCAP, SYNGAP1, ZEB2</td>
<td>27/32 (84%) (4 unknown)</td>
<td>24/36 (67%)</td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td>SACS (2), C5orf42, CLCN1, COL7A1, FBNL5, GAN, GLB1, HIBCH, KIF7, NDUVF1, PEX1, PNPO, POMT2, PRKRA, RAPSN, SLC19A3, STRC, TREC1, WDR19</td>
<td>40 alleles 6 HMZ 14 cmpnd HTZ</td>
<td>20/40 (50%)</td>
</tr>
<tr>
<td><strong>XL</strong></td>
<td>ATRX (2), OFD1 (2), CASK, MECP2, MTM1, PHEX, RBM10, <strong>SMC1A</strong>§</td>
<td>5/10=50% 1 mosaic</td>
<td>4/10 (40%)</td>
</tr>
</tbody>
</table>

* 3 SWI/SNF complex genes for MR

¶ 3 different genes for Noonan

§ 3 different genes for Cornelia de Lange;  ¶ 3 different genes for Noonan
## Cases with Two Molecular Diagnoses

### 7/760

<table>
<thead>
<tr>
<th>Cases</th>
<th>Genes</th>
<th>Diseases</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>SETBP1</em>, <em>CLCN1</em></td>
<td>Schinzel-Giedion midface retraction syndrome, Myotonia congenita</td>
<td>AD, AR</td>
</tr>
<tr>
<td>2</td>
<td><em>TREX1</em>, <em>PHEX</em></td>
<td>Aicardi-Goutieres syndrome, Hypophosphatemic rickets, X-linked dominant</td>
<td>AR, X-linked</td>
</tr>
<tr>
<td>3</td>
<td><em>RAPSN</em>, <em>ABCC9</em></td>
<td>Congenital myasthenic syndrome, Cardiomyopathy dilated type 1O</td>
<td>AR, AD</td>
</tr>
<tr>
<td>4</td>
<td><em>POMT2</em>, <em>SCN2A</em></td>
<td>Muscular dystrophy-dystroglycanopathy, Seizures</td>
<td>AR, AD</td>
</tr>
<tr>
<td>5</td>
<td><em>SMARCA2</em>, <em>SCN1A</em></td>
<td>ID, Coffin Siris, Seizures</td>
<td>AD, AD</td>
</tr>
<tr>
<td>6</td>
<td><em>ATM</em>, <em>AP4M1</em></td>
<td>Ataxia telangiectasia (AT), Spastic paraplegia</td>
<td>AR, AR</td>
</tr>
<tr>
<td>7</td>
<td><em>NF1</em>, <em>MEGF8</em></td>
<td>Neurofibromatosis, type 1, Carpenter syndrome 2</td>
<td>AD, AR</td>
</tr>
</tbody>
</table>
Medically Actionable Mutations Reported

• Strong evidence for pathogenicity and altering management
• Examples of medically actionable mutations
  – Seven patients with FBN1 mutations (Marfan Syndrome)
  – Four patients with mutations in hereditary cancer genes: APC, BRCA2, CDH1, MSH6
  – Three male patients with G6PD mutations
  – Other patients carry mutations mostly in cardiovascular disease genes
Use of WES in Different Clinical Scenarios

- Pediatric
- Adult
- Prenatal
  - DOK7 mutations in case of fetal akinesia
  - NIPBL mutation in case of multiple congenital anomalies
- Pre-conception in case of two previous affected children
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<th>Cases</th>
<th>Genes</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>SLC19A3</em></td>
<td>Biotin- or thiamine-responsive encephalopathy type 2</td>
</tr>
<tr>
<td>2</td>
<td><em>PHOX2B</em></td>
<td>Central hypoventilation syndrome, congenital, with or without Hirschsprung disease (CCHS)</td>
</tr>
<tr>
<td>3</td>
<td><em>ENPP1</em></td>
<td>Arterial calcification of infancy, generalized, type 1 (GACI1)</td>
</tr>
<tr>
<td>4</td>
<td><em>RAPSN</em></td>
<td>Congenital Myasthenic Syndrome</td>
</tr>
<tr>
<td>5</td>
<td><em>DOLK</em></td>
<td>Congenital Myasthenic Syndrome</td>
</tr>
<tr>
<td>6</td>
<td><em>CHRNE</em></td>
<td>Congenital Myasthenic Syndrome</td>
</tr>
<tr>
<td>7</td>
<td><em>SLC25A38</em></td>
<td>Anemia, sideroblastic, pyridoxine-refractory, autosomal recessive</td>
</tr>
<tr>
<td>8</td>
<td><em>TTC37</em></td>
<td>Trichohepatoenteric syndrome 1</td>
</tr>
</tbody>
</table>
### Statistics of WES Reports

#### Focused Report

<table>
<thead>
<tr>
<th>Related Disease-causing</th>
<th>Related VUS</th>
<th>Medically Actionable</th>
<th>*Carrier Status</th>
<th>Pharmacogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>4-9</td>
<td>0-1</td>
<td>0-1</td>
<td>0-4</td>
</tr>
</tbody>
</table>

#### Expanded Report (ordered by 1/3 clients)

<table>
<thead>
<tr>
<th>Unrelated Disease-Causing</th>
<th>Unrelated VUS (AD, 1 hit; AR, 2 hits)</th>
<th>Molecularly deleterious Clinically Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>17-41</td>
<td>17-25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unrelated VUS (AR, 1 hit)</th>
<th>Molecularly Unclassified, Clinically Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>26-64</td>
<td>300-600</td>
</tr>
</tbody>
</table>
Prior Diagnostic Evaluation

- Most cases had extensive prior genetic testing
- CMA, metabolic studies, single gene tests, panels, biopsies
- Suggestion that early use of WES may have cost savings but formal cost-effectiveness studies need to be performed
Example of Previous Evaluation

1 – metabolic screening, karyotype, PWS, brain MRI

2 – VLCFA, muscle bx, respiratory chain, mito DNA, mito depletion panel, PHOX2B, myotonic dystrophy, congenital disorders glycosylation next-gen panel,

3 – CSF neurotransmitters, Cr/guanidinoacetate, urine purines/pyr, NCL, DNA testing for 7 genes – ARX, CDKL5, MECP2
Case 1

- 9.5 yo caucasian M
- H/o several episodes of extreme weakness, spells of apnea requiring intubation, increased respiratory secretions, ptosis, dysphagia, all usually when suffering from a febrile illness. At 8 mo was diagnosed with cardiomyopathy (note says has not recurred)
- FH: Younger sister died when 20 mo (developed febrile illness and stopped breathing), other siblings normal
Case #3

521901

Patient: Age appropriate

- **PHE:** Wt 30th%, Ht 5th%, Ptosis, café au lait spot on right trunk, several small telangiectasias. Normal otherwise.

- **Testing:** muscle bx: ↑ type I fibers (slow), DNA studies: ETF-A, ETF-B, ETFDH (glutaric aciduria II), Acid Maltase (Pompe’s) all normal. Fibroblast enzyme assays: PDH complex, CPT1, CPT2, CACT, CAT, SCAD, MCAD, LCHAD, VLCAD: all normal
Case #1 Compound heterozygous mutation/variant identified in RAPSN

- Gene:  **RAPSN (RECEPTOR-ASSOCIATED PROTEIN OF THE SYNAPSE)**, 11p11.2
  c.872G>A, p.G291D – father is het  
  – Both confirmed by Sanger sequencing
- Congenital Myasthenic Syndrome (CMS), AR
  - Symptoms include bilateral ptosis, weakness of limb, etc. Affects skeletal muscle
  - Frequent exacerbations with respiratory insufficiency provoked by illness/fever/stress
  - Treatment available
Case 1  Possible Second Diagnosis

- **ABCC9** (*ATP-BINDING CASSETTE, SUBFAMILY C, MEMBER 9*), 12p12.1
  - c.4570_4572delTTA_insAAAT, p.V1524fs
  - Mother (age 33) is heterozygous
  - Confirmed by Sanger sequencing

- Cardiomyopathy Dilated Type 10 (CMD10), AD
  - Severely dilated hearts with compromised contractile function and rhythm disturbances
Case 2

• 38 month-old female with static encephalopathy, hypotonia, and seizures
• On Keppra for seizures
• Receives speech, PT and OT
• Physical exam
  – Non-dysmorphic facial features
  – FOC <3rd, length 34th, weight 25th
• Previously evaluated in genetics at 23 and 27 months of age for hypotonia and motor delays
Case 2: Previous work-up

- Ophthalmology
- Neurology x 3
- EEG and a brain MRI (unremarkable)
- Labs (all normal)
  - Thyroid function studies
  - CK
  - Lactate
  - Aldolase
  - Plasma amino acids
  - Acylcarnitine profile
  - Urine organic acids
  - N-glycan and transferrin
  - Very long-chain fatty acids
  - Plasma creatine and guanidinoacetate determination
- CMA- paternally inherited 0.27Mb loss at Xp22.11
- WES was ordered at her third genetics visit
Case 2: WES Results

- De novo heterozygous c.376C>T (p.R126C) mutation in SLC2A1 associated with GLUT1 deficiency syndrome
- Glucose transporter type 1 deficiency syndrome (OMIM #606777)
  - Characterized by infantile-onset seizures, delayed neurologic development, acquired microcephaly, and complex movement disorders, low-normal/low CSF lactate, normal blood glucose, and low CSF glucose
  - Inherited in an AD manner
  - Ketogenic diet is highly effective in controlling seizures and improving the movement disorder and alertness
Case 2: Results Follow-up

- Family was counseled on new diagnosis
- Recurrence risk for future siblings of this patient is low, germline mosaicism cannot be excluded
- AD inheritance reviewed with parents for patient’s future children
- Patient was referred to epilepsy clinic to start ketogenic diet
Case 2: Update

- She’s been on ketogenic diet for ~3 months and is tolerating it well
- Taken off of Keppra, no seizures to date
- Family reports improvement with development
  - More active and alert
  - No longer naps during the day
  - Balance has improved – now rarely falls and is able to make quick turns without falling
  - Improvement in fine motor skills and speech
Summary for WES

- Strong, growing interest in whole exome testing
- Diagnose rare conditions and common conditions
  - Positive rate of 25% in unselected clinical samples
- Early evidence of clinical utility and cost-effectiveness
- Reporting of non-phenotype findings can be challenging
- Expand phenotypic spectrum of many disorders
<table>
<thead>
<tr>
<th>Arthur Beaudet</th>
<th>Richard Gibbs</th>
<th>Jim Lupski</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christine Eng</td>
<td>Donna Muzny</td>
<td>Yaping Yang</td>
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<tr>
<td>Sharon Plon</td>
<td>Jennifer Scull</td>
<td>Jeffrey Reid</td>
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<tr>
<td>Will Parsons</td>
<td>Peter Pham</td>
<td>Alecia Willis</td>
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<tr>
<td>Jeff Mize</td>
<td>Michelle Rives</td>
<td>Alicia Braxton</td>
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<tr>
<td>Yan Ding</td>
<td>Joke Beuten</td>
<td>Sean Kim</td>
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<tr>
<td>Brandon Perthius</td>
<td>Eric Burgess</td>
<td>Fan Xia</td>
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<tr>
<td>Mark Scheel</td>
<td>Neal Niu</td>
<td>Pat Ward</td>
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<td>Nehad Saada</td>
<td>Doreen Ng</td>
<td>Mir Reza Bekheirnia</td>
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<tr>
<td>William Craigan</td>
<td>Megan Landswerk</td>
<td>Magalie Leduc</td>
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<td>Wendy Liu</td>
<td>Richard Person</td>
<td>Alicia Hawes</td>
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Clinical Whole Exome Sequencing (WES) Sign-Out Conference

http://www.bcm.edu/geneticlabs/index.cfm?PMID=21319
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