Role of Clinical Exome Sequencing in Diagnostic Odyssey

Pinar Bayrak-Toydemir, MD, PhD
Professor, Department of Pathology, University of Utah
Medical Director, Molecular Genetics and Genomics, ARUP Laboratories
Outline

- Description of exome sequencing

- Results of our clinical exome cases
  Detection rate based on clinical findings and trio vs proband

- Exome Sequencing interesting case discussions

- Guidelines and Recommendations
Next Generation Sequencing in Molecular Diagnosis

A powerful tool for gene discovery

200 genes are discovered every year

Now a powerful diagnostic tool!

Changed the way we think about scientific approaches in basic, applied and clinical research and diagnostics
Next Generation Sequencing Cost Dropping

Cost per Raw Megabase of DNA Sequence

Moore's Law

National Human Genome Research Institute

http://www.genome.gov/sequencingcosts/
Of approximately \( \sim 19,000 \) protein-coding genes predicted to exist in the human genome, variants that cause Mendelian phenotypes have been identified in \( \sim 3,303 \) genes.

**Dissected OMIM Morbid Map Scorecard (Updated January 9th, 2017):**

<table>
<thead>
<tr>
<th>Class of phenotype</th>
<th>Phenotype</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single gene disorders and traits</td>
<td>4,887</td>
<td>3,303</td>
</tr>
<tr>
<td>Susceptibility to complex disease or infection</td>
<td>699</td>
<td>498</td>
</tr>
<tr>
<td>&quot;Nondiseases&quot;</td>
<td>143</td>
<td>113</td>
</tr>
<tr>
<td>Somatic cell genetic disease</td>
<td>205</td>
<td>117</td>
</tr>
</tbody>
</table>

New 2016 OMIM Disease-Associated Genes

N=179

- Neurological: 72
- Syndromes: 24
- Immunological: 14
- Eye: 11
- Skeletal: 9
- Muscular: 9
- Cardiac: 8
- Mitochondrial: 6
- Metabolic: 5
- Blood: 4
- Reproductive: 4
- Ciliopathies: 3
- Gastrointestinal: 2
- Kidney: 2
- Hearing: 2
- Ectodermal: 2
- Heterotaxy: 1
Exome Sequencing

Sequencing of coding regions of all known genes

- Balanced to cover and obtain full coverage across the medically relevant genes in the human exome

- 100% coverage of all exons in 3,000 of the 4,600 disease associated genes making it the most comprehensive exome sequencing test available
Exome sequencing

- Allows for identification of pathologic variants in newly identified disease genes

- Useful for conditions with locus heterogeneity (long molecular differentials)

- Unexpected/expanded phenotypic variation
Exome Diagnostic Yield in Known Disease Genes in Children

Sawyer et al.

Table 1. Broad phenotypes and associated diagnostic rates in known disease genes of the families studied using WES in FORGE

<table>
<thead>
<tr>
<th>Broad phenotype</th>
<th>Total families (N = 382)</th>
<th>Families with known genes (N = 105)</th>
<th>Diagnostic rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurodevelopmental</td>
<td>98</td>
<td>31</td>
<td>31.6</td>
</tr>
<tr>
<td>Dysmorphic syndromes</td>
<td>80</td>
<td>18</td>
<td>22.5</td>
</tr>
<tr>
<td>Ocular</td>
<td>40</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Metabolic</td>
<td>31</td>
<td>12</td>
<td>38.7</td>
</tr>
<tr>
<td>Neuromuscular</td>
<td>30</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>Ciliopathy</td>
<td>27</td>
<td>12</td>
<td>44.4</td>
</tr>
<tr>
<td>Congenital malformation syndromes</td>
<td>19</td>
<td>4</td>
<td>21.1</td>
</tr>
<tr>
<td>Immunological</td>
<td>17</td>
<td>2</td>
<td>11.8</td>
</tr>
<tr>
<td>Other (isolated cardiac, endocrinology, skeletal dysplasia, connective tissue disorders, mental illness, lung disorder)</td>
<td>20</td>
<td>8</td>
<td>40.0</td>
</tr>
</tbody>
</table>

FORGE, Finding Of Rare Disease GEnes; WES, whole-exome sequencing.

Clinical Sensitivity

Clinical sensitivity may change based on the test ordered and also based on clinical presentation.

- Neurodevelopmental disorders - yield around 73%
- Autism - yield around 28%
- Epilepsy - 30%

(Soden et al, 2014)
(Lee et al., 2014)
(Juusola et al., 2015)
Clinical Sensitivity

De novo variants are reported when both parent’s samples are available for exome sequencing; 35-50% of diagnoses were achieved by identification of de novo variants.

Compound heterozygous/homozygous variants (30%) are reported for autosomal recessive conditions related to the patient’s symptoms.

X-linked mutations are 10%
Diagnostic Yield

Positive, 34%

Negative, 60%

VUS, 6%
Inheritance Pattern Positive Cases

- Dominant de novo: 38, 39%
- Dominant - proband only: 25, 26%
- Dominant inherited: 11, 11%
- X-linked de novo: 10, 10%
- X-linked - mother carrier: 5, 5%
- Homozygous: 5, 5%
- Compound heterozygous: 4, 4%

Courtesy of Tatiana Tvrdik
Proband Only vs Trios Diagnostic Yield

**Proband Only**
- Positive: 25%
- VUS: 6%
- Negative: 69%

**Incomplete Trio**
- Positive: 36%
- Negative: 14, 64%
- VUS: 7%

**Trio**
- Positive: 37%
- Negative: 56%
- VUS: 7%

**Trio Plus**
- Positive: 44%
- Negative: 49%
- VUS: 7%

Courtesy of Tatiana Tvrdik
Power of Trio in Exome Testing

- De novo variants
- Potential to identify parent-of-origin of de novo variants
- Compound heterozygotes and complex variants
- Homozygous vs apparent homozygous variants
- Reduced number of variants to be considered as causative
Diagnostic Yield by Age

Newborn: 46% Positive, 54% Negative
Infant: 39% Positive, 61% Negative
Child: 37% Positive, 63% Negative
Adolescent: 82% Positive, 18% Negative
Adult: 80% Positive, 20% Negative

Courtesy of Tatiana Tvrdik
Causative Disorders

- Syndromes: 34, 37%
- Neurological: 33, 36%
- Muscular: 7, 8%
- Vascular: 5, 5%
- Metabolic: 4, 4%
- Mitochondrial: 4, 4%
- Skeletal: 1, 1%
- Ciliopathy: 1, 1%
- Hearing: 1, 1%
- Gastrointestinal: 1, 1%
- Hematological: 1, 1%

Courtesy of Tatiana Tvrdek
Cases with No Molecular Diagnosis

- Multiple anomalies: 97, 55%
- Neurological: 55, 30%
- Muscular: 5, 3%
- Immunodeficiency: 8, 4%
- Skeletal: 3, 2%
- Gastrointestinal: 3, 2%
- Vascular: 3, 2%
- Ciliary dyskinesia: 1, 1%
- Mitochondrial: 1, 1%
- Failure to thrive: 1, 1%
- Sarcomas: 1, 1%
- Xanthomas: 1, 1%

Courtesy of Tatiana Tvrdik
Limitations of Our Exome Sequencing

The following will not be identified:

- Some coding regions, amenable to capture
- Any genetic changes residing outside of the targeted regions
- Repeat expansions
- Low level of mosaicism
- Structural DNA variation: translocations, inversions, insertions/deletions (indels) and copy number variations
- Mitochondrial genome variants
Case Discussion
CASE 1

**Dysmorphic features**
Narrow palpebral fissures, blepharophimosis, prominent nasolabial folds, small mouth, dimpling on chin, retrognathia and low-set ears

**Distal Arthrogryposis:** finger elbow and knee contractures, ulnar deviation, and fixed thumb adduction, difficulty in opening jaw

**MicroArray:** 409kb gain at 4q32.2

Dave Stevenson, MD, Kathryn Swoboda, MD
# CLINICAL DIFFERENTIAL DIAGNOSIS

<table>
<thead>
<tr>
<th>DISORDER</th>
<th>CLINICAL MANIFESTATION</th>
<th>GENETIC BASIS</th>
<th>TEST RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stuve Wiedmann syndrome (SWS)</td>
<td>Argthrogryposis&lt;br&gt;Long bone bowing&lt;br&gt;Autonomic dysregulation&lt;br&gt;Early death</td>
<td>LIFR&lt;br&gt;Autosomal Recessive</td>
<td>Negative_1&lt;br&gt;Variant VUS</td>
</tr>
<tr>
<td>Freeman Sheldon Syndrome</td>
<td>Face, hands, and feet&lt;br&gt;&quot;whistling face&quot;;&lt;br&gt;chin dimple shaped like an &quot;H&quot; or &quot;V&quot;;&lt;br&gt;malignant hyperthermia</td>
<td>MYH3&lt;br&gt;Autosomal Dominant</td>
<td>Negative_No&lt;br&gt;disease causing mt noted</td>
</tr>
</tbody>
</table>
FILTERING METHODS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon effect</th>
<th>Zygosity</th>
<th>c. dot</th>
<th>p. dot</th>
<th>Pop. Freq. MAF</th>
<th>HGMD &amp; OMIM</th>
<th>dbSNP #</th>
<th>IGV</th>
</tr>
</thead>
<tbody>
<tr>
<td>THEG</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.C677T</td>
<td>p.R193C</td>
<td>0.01</td>
<td>-</td>
<td>rs114537924</td>
<td></td>
</tr>
<tr>
<td>GZMM</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.G283A</td>
<td>p.A65T</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>POLRMT</td>
<td>splicing</td>
<td>Het</td>
<td>c.2641-1G&gt;C</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>FGF22</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.C403T</td>
<td>p.R135C</td>
<td>0</td>
<td>-</td>
<td>rs73507827</td>
<td></td>
</tr>
<tr>
<td>MED16</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.T1979C</td>
<td>p.V660A</td>
<td>0</td>
<td>-</td>
<td>rs45506200</td>
<td></td>
</tr>
<tr>
<td>KISS1R</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.G566A</td>
<td>p.A189T</td>
<td>0.01</td>
<td>HG</td>
<td>rs34615361</td>
<td></td>
</tr>
<tr>
<td>IZUMO4</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.A410T</td>
<td>p.Y137F</td>
<td>0</td>
<td>HG</td>
<td>rs18166606</td>
<td></td>
</tr>
<tr>
<td>TMPS5</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.G392A</td>
<td>p.G79R</td>
<td>0</td>
<td>HG</td>
<td>rs34615361</td>
<td></td>
</tr>
<tr>
<td>ATCAY</td>
<td>splicing</td>
<td>Het</td>
<td>c.647+10C&gt;T</td>
<td>-</td>
<td>0.00</td>
<td>HG</td>
<td>HG</td>
<td></td>
</tr>
<tr>
<td>DAPK3</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.A1193C</td>
<td>p.E388A</td>
<td>0</td>
<td>HG</td>
<td>HG</td>
<td></td>
</tr>
<tr>
<td>SH3GL1</td>
<td>splicing</td>
<td>Het</td>
<td>c.188-3C&gt;T</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SAFB2</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.A1369G</td>
<td>p.T457A</td>
<td>0.01</td>
<td>rs61174936</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SLC25A1</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.C78G</td>
<td>p.L26V</td>
<td>rs117420388</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>KANK3</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.C1924T</td>
<td>p.L642F</td>
<td>rs142931419</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MUC16</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.T11440C</td>
<td>p.S3814F</td>
<td>rs145106176</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C1orf38</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.G467A</td>
<td>p.R156Q</td>
<td>rs376886396</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACP5</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.G163A</td>
<td>p.A65T</td>
<td>0</td>
<td>HG</td>
<td>rs35614788</td>
<td></td>
</tr>
<tr>
<td>TNPO2</td>
<td>splicing</td>
<td>Het</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>HG</td>
<td>HG</td>
<td></td>
</tr>
<tr>
<td>HOOK2</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.C2042T</td>
<td>p.A861V</td>
<td>0</td>
<td>HG</td>
<td>HG</td>
<td></td>
</tr>
<tr>
<td>NOTCH3</td>
<td>nonsyn</td>
<td>Het</td>
<td>c.A2933C</td>
<td>p.S978R</td>
<td>0.00</td>
<td>HG</td>
<td>rs141956294</td>
<td></td>
</tr>
</tbody>
</table>

**Gene details**
- **Summary:** None
- **OMIM Disease:** None
- **Inheritance pattern:** None
- **Phenotypes:** None

**HGMD & OMIM**
- **No disease filters set.**
TWO LIFR VARIANTS ON DIFFERENT CHROMOSOMES

c.46G>A; p.Asp16Asn

2688-71G>A
DONOR SPLICING SITE

LIFR c.2668-71G>A

WT

c.2668-71G>A

Splice site predictions for 1 sequence with donor score cutoff 0.10, acc

<table>
<thead>
<tr>
<th>Start</th>
<th>End</th>
<th>Score</th>
<th>Exon</th>
<th>Intron</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>106</td>
<td>0.42</td>
<td>tctgcaggttgctt</td>
<td></td>
</tr>
<tr>
<td>253</td>
<td>250</td>
<td>0.16</td>
<td>acagaaatgtgagta</td>
<td></td>
</tr>
<tr>
<td>354</td>
<td>368</td>
<td>0.14</td>
<td>gctggggtagagct</td>
<td></td>
</tr>
<tr>
<td>370</td>
<td>384</td>
<td>0.78</td>
<td>gattaagttgagca</td>
<td></td>
</tr>
<tr>
<td>435</td>
<td>449</td>
<td>0.25</td>
<td>gacagctgtggcaca</td>
<td></td>
</tr>
<tr>
<td>444</td>
<td>458</td>
<td>0.59</td>
<td>gacagctgtggtggt</td>
<td></td>
</tr>
<tr>
<td>471</td>
<td>485</td>
<td>0.44</td>
<td>gtaacgggttagaga</td>
<td></td>
</tr>
</tbody>
</table>

Splice site predictions for 1 sequence with donor score cutoff 0.10, acc

<table>
<thead>
<tr>
<th>Start</th>
<th>End</th>
<th>Score</th>
<th>Exon</th>
<th>Intron</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>106</td>
<td>0.42</td>
<td>tctgcaggttgctt</td>
<td></td>
</tr>
<tr>
<td>253</td>
<td>250</td>
<td>0.16</td>
<td>acagaaatgtgagta</td>
<td></td>
</tr>
<tr>
<td>354</td>
<td>368</td>
<td>0.14</td>
<td>gctggggtagagct</td>
<td></td>
</tr>
<tr>
<td>370</td>
<td>384</td>
<td>0.78</td>
<td>gattaagttgagca</td>
<td></td>
</tr>
<tr>
<td>435</td>
<td>449</td>
<td>0.25</td>
<td>gacagctgtggcaca</td>
<td></td>
</tr>
<tr>
<td>444</td>
<td>458</td>
<td>0.80</td>
<td>gacagctgtggggt</td>
<td></td>
</tr>
<tr>
<td>471</td>
<td>485</td>
<td>0.44</td>
<td>gtaacgggttagaca</td>
<td></td>
</tr>
</tbody>
</table>
REPORTING OF LIFR

• Gene: LIFR (NM_002310)

• Variant:
  – c.46G>A; p.Asp16Asn (one copy) - Variant of Uncertain Significance
  – c.2336-71G>A (one copy) - Variant of Uncertain Significance

• Inheritance pattern: Autosomal recessive
DE NOVO MUTATION ?
DE NOVO MUTATION: NALCN GENE

c.1768C>T; p.Leu590Phe
Na⁺, K⁺, and Ca(2+) • Mainly expressed in CNS • Synapse development and synaptic density (Lu et al., 2007) • KO mice: die of respiratory rhythm

NALCN (Cochet-Bissuel 2014)

Courtesy of Eric Bend and Erik Jorgensen
**NALCN mutations result in:**
Infantile hypotonia with psychomotor retardation and facial dysmorphism (IHPRF; MIM #615419)

<table>
<thead>
<tr>
<th>LOSS-OF-FUNCTION MUTATION</th>
<th>GAIN-OF-FUNCTION MUTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal recessive inheritance</td>
<td>Putative dominant inheritance ?</td>
</tr>
<tr>
<td>Mild to severe hypotonia</td>
<td>Hypertonia - distal contractures ?</td>
</tr>
<tr>
<td>Viable</td>
<td>Infant mortality ?</td>
</tr>
</tbody>
</table>

![Image of NALCN protein structure](image)

[Al-Sayed et al. J Hum Genet 2013](Citation)
[Koroglu et al. J Med Genet 2013](Citation)

Courtesy of Eric Bend and Erik Jorgensen
THE C-TERMINUS OF THE S6 IS CRITICAL FOR CHANNEL GATING

Human variant from clinical exome (L590F)

Courtesy of Eric Bend and Erik Jorgensen
DOES THE NALCN SNP CAUSE A GAIN-OF-FUNCTION CHANNELOPATHY?

Predictions

- Dominant inheritance ✓
- Hypertonia ✓
- Increased neurotransmission □

Courtesy of Eric Bend and Erik Jorgens
THE HUMAN SNP PHENOCOPIES A GAIN-OF-FUNCTION NCA-1 ALLELE

Wild Type

V637F
D647E
V637F/+ 

D647E/+ 

Wild Type

V637F
D647E
V637F/+ 2 mm

○ Wild Type
◆ Gain-of-Function
● Human SNP

Courtesy of Eric Bend and Erik Jorgensen
De novo mutations in NALCN cause a syndrome characterized by congenital contractures of the limbs and face, hypotonia, and developmental delay.


NALCN channelopathies: Distinguishing gain-of-function and loss-of-function mutations.

Bend EG, Si Y, Stevenson DA, Bayrak-Toydemir P, Newcomb TM, Jorgensen EM, Swoboda KJ.
CASE 2

Public database filtering
CASE 2:

a 7-year-old boy of hispanic/native american/caucasian ancestry

Clinical Findings:
Pre and postnatal overgrowth,
Moderate ID,
Not typical Sotos face,
Advanced bone age,
History of laryngomalacia,
Hypotonia,
No history of seizure,
Mild optic nerve hypoplasia

Other Testing Results:

MRI showed a small optic chiasm, focal encephalomalacia or dilated perivascular spaces.
The patient had a normal genomic microarray.

John Carey, MD
EXOME DATA

Variants in targeted genes: 56,890

Variants: 1,738

Variants: 1,582

Exclude parent homozygous

Compound heterozygous or homozygous variants: 20

Hemizygous variant shared with mom on X chr: 25

Variants in HGMD/OMIM located on exons or junction +/-10: 461

De novo: 3
1 FBN1
1 DNMT3A
1 TRAM2

Subtract common variant of frequency >1% and internal frequency 3%

Exclude intergenic, 5’and 3’ UTRs, and noncoding RNA

AR, X-linked

AD
De Novo Variant: FBN1  c.4894C>T,p.Arg1632Cys

This FBN1 variant (p.Arg1632Cys) alters a moderately conserved amino acid and creates an extra cysteine residue between cysteine residues 4 and 5 (Cys1631 and Cys1633) in the EGF-like calcium-binding domain 27.

FBN1 protein contains 47 epidermal growth factor (EGF)-like domains which are characterized by six conserved cysteine residues. These six cysteine residues form three disulfide bonds that are critical for the normal protein structure of FBN1.

Cysteine substitutions that disrupt one of the three disulfide bonds are frequent causes of Marfan syndrome.
De Novo Variant: $FBN1$ c.4894C>T, p.Arg1632Cys

Computational analyses predict that this $FBN1$ variant (p.Arg1632Cys) will affect protein function (SIFT: deleterious, MutationTaster: disease causing, PolyPhen-2: probably damaging).

In addition, it is only reported in one individual in the Exome Aggregation Consortium database (1 out of 121378 alleles).

Although this particular $FBN1$ variant (p.Arg1632Cys) has not been reported in the literature, a different amino acid alteration at the same codon (p.Arg1632His) has been reported in a patient that met Ghent criteria for Marfan syndrome with ocular findings and no skeletal or cardiovascular findings.
SECOND DE NOVO VARIANT

*602769
DNA METHYLTRANSFERASE 3A; DNMT3A

HGNC Approved Gene Symbol: DNMT3A

Cytogenetic location: 2p23.3 Genomic coordinates (GRCh38): 2:25,232,960-25,342,589 (from NCBI)

<table>
<thead>
<tr>
<th>Location</th>
<th>Phenotype</th>
<th>Phenotype MIM number</th>
<th>Inheritance</th>
<th>Phenotype mapping key</th>
</tr>
</thead>
<tbody>
<tr>
<td>2p23.3</td>
<td>Tatton-Brown-Rahman syndrome</td>
<td>615879</td>
<td>AD</td>
<td>3</td>
</tr>
</tbody>
</table>
TATTON-BROWN-RAHMAN SYNDROME; TBRS

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>SUBCATEGORY</th>
<th>FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>-</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Growth</td>
<td>Height</td>
<td>Tall stature (+3 S.D)</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>Head</td>
<td>Large head circumference (+2.5 SD)</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>Round face [EoM image]</td>
</tr>
<tr>
<td></td>
<td>Eyes</td>
<td>Heavy horizontal eyebrows</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Narrow palpebral fissures</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Heart</td>
<td>Atrial septal defect (less common)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>External Features</td>
<td>Umbilical hernia (less common)</td>
</tr>
<tr>
<td>Skeletal</td>
<td>Spine</td>
<td>Scoliosis (less common)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Central Nervous System</td>
<td>Intellectual disability, mild to moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seizures (less common)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>-</td>
<td>All reported cases result from de novo mutation (last curated July 2014)</td>
</tr>
<tr>
<td>Molecular Basis</td>
<td>-</td>
<td>Caused by mutation in the DNA methyltransferase 3A gene (DNMT3A, 602769.0001)</td>
</tr>
<tr>
<td>CATEGORY</td>
<td>SUBCATEGORY</td>
<td>FEATURES</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Inheritance</td>
<td>-</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Growth</td>
<td>Height</td>
<td>Tall stature (+3 S.D)</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>Head</td>
<td>Large head circumference (+2.5 SD)</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>Round face [PoM image]</td>
</tr>
<tr>
<td></td>
<td>Eyes</td>
<td>Heavy horizontal eyebrows</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Narrow palpebral fissures</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Heart</td>
<td>Atrial septal defect (less common)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>External Features</td>
<td>Umbilical hernia (less common)</td>
</tr>
<tr>
<td>Skeletal</td>
<td>Spine</td>
<td>Scoliosis (less common)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Central Nervous System</td>
<td>Intellectual disability, mild to moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seizures (less common)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>-</td>
<td>All reported cases result from de novo mutation (last curated July 2014)</td>
</tr>
<tr>
<td>Molecular Basis</td>
<td>-</td>
<td>Caused by mutation in the DNA methyltransferase 3A gene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(DNMT3A, 602769.0001)</td>
</tr>
</tbody>
</table>
Characteristic facial appearance in DNMT3A overgrowth syndrome

Tatton-Brown et al. Nat Genet 2014
Pathogenic **DNMT3A variant** is present in a “normal” population database
Information about 66 individuals from ExAC Database

- Median age 60
- Age range 55-80
- Allelic ratio: Ranges 10-48%
- 11 individuals—Somatic data from cancer tissue
- 55 individuals- Germline data
DNMT3A: c. 2645G>A, p.Arg882His

It affects the highly conserved methyltransferase domain and reduces methyltransferase activity by approximately 80% compared to the wild type protein, which results in focal hypomethylation at specific CpG sites throughout genome.
Somatic DNMT3A variants are commonly found in patients with hematologic malignancies and in patients with age-related clonal hematopoiesis without overt disease but with increased risk for subsequent development of a hematologic malignancy.

The p.Arg882His variant is the most common somatic variant of DNMT3A observed in patients with age-related clonal hematopoiesis or hematologic malignancies.
Next Generation Sequencing

Next Generation Sequencing (NGS) incorporates two processes: (1) the analytical wet bench process of sample and library preparation and sequence generation and (2) the bioinformatics process or pipeline of sequence alignment, annotation and variant calling. These two processes are inextricably linked as the output from each process supports the optimization of the other. The large volumes of data produced by NGS platforms put substantial demands on laboratories in terms of the requirements for documentation, validation, quality control and assurance, monitoring, data storage, as well as assessment and implementation of new technology and software releases.

Inspector Instructions:

- Sampling of next generation sequencing policies and procedures
- Records of wet bench processing and bioinformatics process validation
- QM program records with corrective action for component failure
- Sampling of exception log records
Guide validation of samples, analysis and reporting

Clinical Laboratory Standards for Next Generation Sequencing

Heidi L. Rehm, PhD1,2, Sherri J. Bale, PhD3, Pinar Bayrak-Toylumir, PhD4, Jonathan S. Berg, MD5, Kerry K. Brown, PhD6, Joshua L. Deignan, PhD7, Michael J. Erle, PhD8, Birgit H. Funke, PhD1,2, Madhura R. Hegde, PhD8, Elaine Lyon, PhD9; A Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee

Assuring the Quality of Next-Generation Sequencing in Clinical Laboratory Practice

Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) Workgroup Principles and Guidelines

Supplementary Guidelines
“Direct laboratories to return with each genomic sequencing order results from 57 genes in which mutations greatly increase risk of 24 serious, but treatable diseases, even if clinicians do not suspect patients have them.”
“Direct laboratories to return with each genomic sequencing order results from 57 genes in which mutations greatly increase risk of 24 serious, but treatable diseases, even if clinicians do not suspect patients have them.”
What are incidental (or secondary) findings?

Variants found by exome/genome sequencing, which are unrelated to the disease of interest

- majority of them are benign
- a small number of them (between 1-5) might be well-described, disease-associated mutations
Incidental Findings

The ACMG Working Group recommended that the laboratory **actively search** for the specified types of mutations in the specified genes listed in these recommendations.

Mandatory reporting known mutations for the disorders:
- Hereditary cancers,
- Marfan syndrome,
- Long QT syndrome,
- Brugada syndrome,
- Certain cardiomyopathies
Returning incidental findings in children

“Recommendations for seeking and reporting incidental findings not be limited by the age of the person being sequenced.

The ethical concerns about providing children with genetic risk information about adult-onset diseases were outweighed by the potential benefit to the future health of the child and the child’s parent of discovering an incidental finding where intervention might be possible.”
Patient Consent and Opt-in/out option

- Proband and family members need to consent for exome sequencing and incidental finding

- the ACMG Working Group revised document offers the patient a preference as to whether or not to receive the minimum list of incidental findings described in these recommendations.
Around 90% of cases would like to receive secondary findings

ARUP secondary finding frequency is 1-2%
Conclusion

- Clinical exome sequencing is effective to diagnosis heterogeneous disorders, non-specific or atypical presentation, especially for neurological and neuromuscular disorders.

- Sensitivity depends on:
  - Medical Exome enrichment
  - Including intronic regions and promoter regions to our bed file
  - Collaboration with clinicians
  - Follow up functional studies

- Quality control measures, data analysis and reporting of incidental findings will continue to evolve and improve.