Clinical Exome Sequencing at GeneDx

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Licensed Genetic Counselor
GeneDx, Gaithersburg, MD
Disclosure

- I am an employee of GeneDx, a clinical genetic testing laboratory that develops and performs whole exome sequencing.
Exome sequencing in the lay press

The New York Times
Health

DNA Test for Rare Disorders Becomes More Routine

TIME
Want to Know My Future?

New genetic tests can point to risks—but not always a cure
BY BONNIE ROCHMAN
The Exome

- The coding region of the genome = ~180,000 exons
- ~1-2% of the genome (30Mb)
- ~20,500 genes
- 85% of mutations known to cause disease are in exons
- The most common mutations being found in exome sequencing are *de novo* dominant changes
- On average, there is one de novo variant per exome per generation! (and 74 de novo variants per genome)
# Single gene sequencing tests and panels vs. WES: Technical Considerations

<table>
<thead>
<tr>
<th><strong>Targeted Sequencing Panel/single gene</strong></th>
<th><strong>Whole Exome Sequencing</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes are selected for their relationship to a particular phenotype</td>
<td>All genes included in exome sequencing</td>
</tr>
<tr>
<td>Requires PCR</td>
<td>No PCR</td>
</tr>
<tr>
<td>Requires protocol optimization</td>
<td>Target sequences “captured” usually using a kit</td>
</tr>
<tr>
<td>Can be “tweaked” to be sure to get every exon</td>
<td>No “tweaking” available; loss of sensitivity</td>
</tr>
<tr>
<td>Good sequence quality – read depth of 10-20X</td>
<td>Requires average read depth of 80-100X</td>
</tr>
<tr>
<td>Confirmation by orthologous method</td>
<td>Confirmation by orthologous method</td>
</tr>
<tr>
<td>Takes time to add new genes to panel</td>
<td>Very flexible; can analyze any gene</td>
</tr>
</tbody>
</table>
### Single gene sequencing tests and panels vs. WES: Clinical Considerations

<table>
<thead>
<tr>
<th><strong>Targeted Sequencing Panel/single gene</strong></th>
<th><strong>Whole Exome Sequencing</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Can get full/nearly full coverage of all genes of interest</td>
<td>May get incomplete coverage of some genes of interest</td>
</tr>
<tr>
<td>May need to supplement with other technology (e.g. microarray for CNVs)</td>
<td>Quickly improving at CNV detection</td>
</tr>
<tr>
<td>Fewer VOUS</td>
<td>Likelihood of many VOUS</td>
</tr>
<tr>
<td>Limited number of genes</td>
<td>Nearly unlimited number of genes (~95% coverage of ~20,000 genes)</td>
</tr>
<tr>
<td>Higher cost</td>
<td>Cost control</td>
</tr>
<tr>
<td>Incidental findings limited to specific genes evaluated</td>
<td>Incidental or secondary findings may include conditions not associated with the diagnosis; ACMG recs</td>
</tr>
<tr>
<td>Better test when phenotype is known to be associated with genes in a panel</td>
<td>Good test when there are many conditions in the differential diagnosis</td>
</tr>
</tbody>
</table>
Today’s diagnostic odyssey

- Parents Matt Might and Cristina Casanova have a son who – after three years of testing – was diagnosed by WES as the first person with mutations in the NGLY1 gene (N-glycanase 1 deficiency; a congenital disorder of glycosylation).

- Story is told by father in a fantastic blog post: Hunting Down My Son’s Killer (matt.might.net)
Bertrand Might’s diagnostic odyssey

- Born in 2008 and diagnosed with motor delays, severe cognitive impairment, seizures, liver dysfunction, diminished reflexes, alacrima
- At different times, suspected ataxia-telangiectasia, inborn error of metabolism (NOS), Allgrove syndrome, male Rett syndrome, Schinzel-Giedion, an X-linked condition (ruled out by testing maternal grandfather), vitamin deficiency (mom’s dx)
- Need et al. (2012) did WES on the trio and found that Matt and Cristina were heterozygous (carriers) of mutations in the NGLY1 gene and Bertrand had inherited both mutations.
  - NGLY1 is critical to the endoplasmic reticulum-associated pathway, which degrades misfolded proteins
  - Looks very promising as the cause, but still a candidate gene – not diagnostic without additional patients showing the same thing
Families Connecting

- Matt’s blog post caught the attention of other families and providers, connecting families from all over the world
  - Grace, who had exome sequencing at Baylor where they found NGLY1 mutations. After reading the blog post, the geneticist asked about Grace’s tear production – she had very little.
  - Siblings in Israel
  - A German family living in India with an affected son; diagnosed by enzyme assay prior to sequencing
  - Two siblings who were in a WES study at Yale
  - Two sisters in Georgia who were misdiagnosed with CP
Mutations in \textit{NGLY1} cause an inherited disorder of the endoplasmic reticulum–associated degradation pathway

The shifting model in clinical diagnostics: how next-generation sequencing and families are altering the way rare diseases are discovered, studied, and treated

Matthew Might PhD & Matt Wilsey MBA

We are the fathers of two patients with a newly diagnosed syndrome that is highlighted in the study by Enns et al.\textsuperscript{1} Our children are two among a handful of others in the world with this disease caused by mutations in the \textit{NGLY1} gene. It is the first recognized disorder...
Connecting providers

GeneXome Analyzer

Details for NGLY1

N-glycanase 1

Aliases: PNG1, FLJ11005

Gene information last updated 07/27/2014

Activity Log

GeneXome Synopsis:

The enzyme N-glycanase 1 (NGLY1) is involved in the degradation of misfolded glycoproteins. Deficiency of NGLY1 leads to an autosomal recessive congenital disorder of glycosylation characterized by hypolactacina or alkalactia, intractable seizures, developmental delay, microcephaly, involuntary movements, hypotonia, diminished reflexes, and liver dysfunction (Enns et al., 2014, Need et al., 2012).

Last updated 2014-07-15 09:25:29 by ablesson9459

References:

Enns et al. (2014) Genetics in medicine: official journal of the American College of Medical Genetics; (PMID: 24651605); Need et al. (2012) Journal of medical genetics; 49 (6):355-61 (PMID: 22581936);

Last updated 2014-04-10 14:47:07 by sdward9516

Description:

Specifically deglycosylates the denatured form of N-linked glycoproteins in the cytoplasm and assists their proteasome-mediated degradation. Cleaves the beta-aspartyl-glucosamine (GlbHAc) of the glycan and the amide side chain of Asn, converting Asn to Asp. Prefers proteins containing high mannose over those bearing complex type oligosaccharides. Can recognize misfolded proteins in the endoplasmic reticulum that are exported in the cytosol to be destroyed and deglycosylate them, while it has no activity toward native proteins. Deglycosylation is prerequisite for

Search the Web

Lists

Observations

Pubmed
OSCAR
Google Scholar
HGMD
OMIM
ClinVar
Gene Distiller
Gene Tests
GeneCard
GeneDeck
Genic Intolerance
Wikipedia

ALL
Encephalopathy(OMIM_200010)
GeneX
Glossary(OMIM_200010)
HGMD-2014-1
HGMD-2014-1 Disease
HGMD_2014_2
HGMD_Disease_2014_2
Neuropathy(OMIM_201400)
OMIM

FAM131414 - Y
FAM131414 - Y
FAM131548 - Y
FAM131548 - Y
FAM140122 - Y
FAM140122 - Y

3 Patients

You are logged in as Cheryl Scacheri. Log out

You have successfully logged in

+ Add Comment
Data Sharing: ClinVar, PROMPT
<table>
<thead>
<tr>
<th>Disease (inheritance)</th>
<th>Gene</th>
<th>No. of Studied families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blepharophimosis-ptosis-intellectual-disability syndrome (AR)</td>
<td>UBE3B</td>
<td>Three families</td>
</tr>
<tr>
<td>Hereditary spastic paraparesis (AR)</td>
<td>TECPR2</td>
<td>Three Jewish Bukharian families</td>
</tr>
<tr>
<td>Intellectual-disability syndrome (AD)</td>
<td>PACS1</td>
<td>Two individuals</td>
</tr>
<tr>
<td>Shprintzen-Goldberg syndrome (AD)</td>
<td>SKI</td>
<td>One individual</td>
</tr>
<tr>
<td>Hyperphosphatasia with mental retardation syndrome (AR)</td>
<td>PIGO</td>
<td>Two individuals</td>
</tr>
<tr>
<td>Acrodysostosis (AD)</td>
<td>PDE4D</td>
<td>Two individuals</td>
</tr>
<tr>
<td>Wiedemann-Steiner syndrome (de novo)</td>
<td>MLL</td>
<td>Five individuals</td>
</tr>
<tr>
<td>Dyggve-Melchior-Clausen syndrome (AR)</td>
<td>RAB33B</td>
<td>One family</td>
</tr>
<tr>
<td>Coffin-Siris syndrome (de novo)</td>
<td>ARID1B</td>
<td>Three individuals</td>
</tr>
<tr>
<td>Coffin-Siris syndrome (de novo)</td>
<td>SMARCB1</td>
<td>Five individuals</td>
</tr>
<tr>
<td>Intellectual disability with neuronal migration defects (de novo)</td>
<td>DYNC1H1</td>
<td>One family</td>
</tr>
<tr>
<td>Baraitser-Winter syndrome (de novo)</td>
<td>ACTB and ACTG1</td>
<td>Three families</td>
</tr>
<tr>
<td>X-linked Joubert syndrome (X-linked)</td>
<td>OFD1</td>
<td>One family</td>
</tr>
<tr>
<td>Genitopatellar syndrome (de novo)</td>
<td>KAT6B</td>
<td>Five individuals; three individuals</td>
</tr>
<tr>
<td>Floating-Harbor syndrome (de novo)</td>
<td>SRCAP</td>
<td>Five individuals</td>
</tr>
<tr>
<td>Myhre syndrome (de novo)</td>
<td>SMAD4</td>
<td>One individual; 11 individuals</td>
</tr>
<tr>
<td>Sjögren-Larsson syndrome (AR)</td>
<td>ELOVL4</td>
<td>Two individuals</td>
</tr>
<tr>
<td>Say-Barber-Biesecker-Young-Simpson syndrome (de novo)</td>
<td>KAT6B</td>
<td>Three individuals</td>
</tr>
<tr>
<td>KBG syndrome (AD)</td>
<td>ANKRD11</td>
<td>Two families</td>
</tr>
<tr>
<td>3-M syndrome (AR)</td>
<td>CCDC8</td>
<td>Three families</td>
</tr>
</tbody>
</table>
Limitations of Capture

- Disorders due to large repeat expansions (FraX, HD) will not be identified* Need to test for these using adjunct method
- CNVs, at this time, are still best identified using arrayCGH methods*
- Not all nucleotides of all genes will be covered
- Pseudogenes and homologous regions may also be captured. This may reduces the sensitivity
- Sequencing multiple family members can compensate for low coverage when a variant is observed in multiple family members (Trios are usually best: proband and both parents).

*Also a limitation of Sanger and next gen sequencing
Patients who have undergone an extensive diagnostic odyssey, with no molecular basis identified

Patients with a known or suspected disorder that could be explained by one of many genes

Patients with intellectual disability, congenital anomalies (including POC), congenital heart defects

Patients with a likely genetic condition, but clinical testing doesn’t exist or has limitations
Exome Process Flow

1. **Family’s samples arrive**
   - **Abstract clinical information**
   - **Exome Capture (Agilent)**
   - **Exome Sequencing (Illumina)**

2. **Exome Sequencing**
   - **Primer design**
   - **Selection of relevant variants to confirm by Sanger**
   - **Medical review of Exome Data in the XA**
   - **BiInformatics (alignment, SNP-calling) Upload to XA**

3. **Medical review: Secondary findings**
   - **Secondary findings discussion**

4. **Technical Review (Review Analysis)**
   - **Report Writing**
   - **Director Review And Signature**
   - **Reporting**
### PCR, capillary sequencing and analysis

- Polymerase chain reaction used to amplify region of interest
- 2 reads, one in each direction
- Sanger (capillary or di-deoxy) sequencing
NextGen Library Preparation
Used for XomeDx and Panels

- Patient DNA is fragmented, amplified and prepared for automated sequencing
  - Fragments are modified with: Adapters, Sequencing Primer Oligos and Index sequences ligated to each end.
  - Index: “bar code” allows multiplexing of many samples/lane
Preparation for Sequencing: Cluster Generation

- Flow cells have 8 lanes
- “Lawn” of oligos that bind to adapters
- Undergo extensions and bridge amplifications
- Generates unique DNA clusters
- Sequencing primer is hybridized to the DNA templates
Massively Parallel Sequencing

- Clusters are sequenced simultaneously
- “Sequencing by synthesis”
- Bases added one at a time
- After each base is added, the clusters are excited by a laser and emit a color that is captured, and another base is added
Capturing and Sequencing the Exome

BioInformatics

Reads are aligned to reference sequence
- Reads are 100 bases, which is shorter than most PCR amplicons used in Sanger sequencing

Coverage depth is the number of reads per position
- Sanger sequencing generally looks at 2 reads: forward and reverse
- Coverage varies from zero to hundreds of reads
- Like to have 10x coverage and good quality data to call a base change

Sequencing reads

Human reference sequence
Analysis Pipeline and Variant Caller

Het 3bp del
Het SNV

Single base changes
Copy number variants
Heterozygosity
Homzygosity
Hemizygosity
Human reference sequence
How is exome data analyzed at GeneDx?

In-house analysis tool to identify sets of genes and variants relevant to the phenotype

Analysis (patient with clefting and intellectual disability)

<table>
<thead>
<tr>
<th>Lists</th>
<th>Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associated with Gene List</td>
<td>Proband (1224292) is Select... with &gt;=20 quality</td>
</tr>
<tr>
<td>Cleft_lip_palate</td>
<td>(+ (De Novo) (Hom Recessive) (Compound Het) (X-Linked Recessive))</td>
</tr>
</tbody>
</table>

Combined Gene List (130)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Inherit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCD3, ACTB, ALX3, ASXL1, ATR, B3GALT1, B3GAT3, BCOR, BMP4, BMPER, BRAF, BUB1B, CHD7, CHRNA1, CHRND, CHRNG, COL11A1, COL11A2, COL2A1, CTNND2, DHCRC24, DHCRC7, DOK7, DYNC2H1, EFNB1, ESCO2, EVC, EVC2, EYA1, FAM123B, FAM20C, FGD1, FGF8, FGR1, FGFR2, FGFR3, FLNA, FLNB, FOXC2, FOXE1, FOXF2, FRAS1, FREM2, FTO, G6PC3, GDF6, GJA1, GLI2, GLI3, CPC3, HOXA2, HYAL1, HYLS1, ICK, IRF6, JAG2, KCNJ2, KIF7, KRAS, LBR, LHX8, LMNA, LMX1B, MAP2K1, MAP2K2, MED12, MID1, MKS1, MLL2, MSX1, MSX2, NBN, NEB, NEK1, NIPBL, OFD1, ORC1, PAX3, PAX9, PDGF, PEX2, PEX7, PHF8, PIGV, POMT1, PORCN, PQBP1, PROK2, PRRX1, PTCH1, PTEN1, PVRL1, RAI1, RAPSN, RB1, RBM10, RECL, RPRP1L1, RPS19, RUNX2, SATB2, SCARF2, SEMA3E, SEPT9, SHH, SIX3, SKI, SLC26A2, SLC35D1, SMOC1, SMS, SOX9, SPRY2, TBX1, TBX10, TBX15, TBX22, TBX4, TCOF1, TAFAP2A, TGFBI1, TGFBR1, TGFBR2, TGFIF1, TP63, TWIST1, UBB, WNT3, ZEB2, ZMPSTE24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COL11A1</td>
<td>splicing</td>
<td>AR, AD</td>
</tr>
<tr>
<td>DYNC2H1*</td>
<td>missense</td>
<td>AR</td>
</tr>
<tr>
<td>DYNC2H1*</td>
<td>missense</td>
<td>AR</td>
</tr>
<tr>
<td>DYNC2H1*</td>
<td>missense</td>
<td>AR</td>
</tr>
<tr>
<td>EVC2</td>
<td>splicing</td>
<td>AR, AD</td>
</tr>
<tr>
<td>FAM20C</td>
<td>missense</td>
<td>AR</td>
</tr>
<tr>
<td>FLNB</td>
<td>splicing</td>
<td>AR, AD</td>
</tr>
<tr>
<td>FOXE1</td>
<td>nonfs</td>
<td>AR, AD</td>
</tr>
</tbody>
</table>

NM_001164318:ex1:1.c.1611-4G>A
NM_001164319:ex1:1.c.1611-4G>A
NM_001164317:ex1:1.c.1611-4G>A
NM_004473:ex1:c.532_537del.p.178_179del
# Analysis

## Gene List

Filter by de novo variants

Frameshift variant identified

### Lists

- Associated with: Gene List
- Cleft_lip_palate

(Add Filter) | (View Combined Gene List)

### Comparisons

- **Proband (1224292)** is **Not Wildtype** with >=20 quality
  - And **Mother (1224293)** is **Wildtype** with >=1 quality (- remove)
  - And **Father (1224294)** is **Wildtype** with >=1 quality (- remove)

(De Novo) (Hom Recessive) (Compound Het) (X-Linked Recessive)

Add Filter

### Showing 1 of 1 entries

<table>
<thead>
<tr>
<th>Flag</th>
<th>Gene</th>
<th>Proband(M)</th>
<th>Mother</th>
<th>Father</th>
<th>Annotations</th>
<th>Type</th>
<th>Inher</th>
<th>Position</th>
</tr>
</thead>
</table>

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Useful links:
- [HGMD disease, Cleft_lip_palate](#)

**Update Results** | **Save Search** | **Clear All**
Reporting

- For proband/trio – a report is written for the proband only (other relatives used for segregation)

- Analysis is phenotype-driven and report reflects that mindset:
  - Cat 1. Variants in gene definitely associated with phenotype
  - Cat 2. Variants in gene possibly associated with phenotype
  - Cat 3. Variants in candidate gene, with evidence supporting possible association with phenotype (always as VUS)
  - Cat 4. Pathogenic variant in gene not known to be associated with phenotype, but with important clinical relevance. Reported only AFTER discussion with referring MD/GC
Reported Findings and Classification of Results

Results

- **POSITIVE**: known pathogenic and expected pathogenic variants in genes known to be associated with the phenotype
  - See Interpretation: known/expected pathogenic variants and VUS in genes possibly associated with the phenotype, incl. candidate genes (rarely)
    - May need follow up with different test method
    - May not completely fit clinical picture of genetic disorder associated with gene
    - Patients with similar phenotypes and mutations may lead to novel disease genes

- **Negative**: No variants identified to be associated with clinical phenotype

Plus, we find mitochondrial gene mutations 3% of the time that we do mtDNA sequencing in addition to WES
Our Experience – The samples

- > 10,000 exomes sequenced (total)
  - Includes >4,500 healthy controls
- 788 completed cases (families) from December 2012 to November 2013 (Presented at ACMG meeting in Nashville)
- >75% of cases had proband plus at least one relative
- During this one-year period a Definitive Diagnosis was made in 31% of trios (158/513)
- Proband only: 28.8% positive overall; 44% in cases with targeted analysis.
GeneDx Experience – The Mutations

45% of definitive cases were *de novo* (87% dominant and 13% X-linked)
Some XomeDx Definitive Diagnoses

>10,000 exomes  ~32% of cases result in a definitive diagnosis

- **ABHD12** compound heterozygous mutations in Polyneuropathy, Hearing loss, Ataxia, Retinitis pigmentosa and Cataract (PHARC)
- **EP300** mutation in Rubinstein-Taybi syndrome (N=2)
- Homozygous **SGSH** mutation in Sanfilippo A syndrome (mucopolysaccharidosis IIIA)
- **TCF4** mutation in Pitt-Hopkins syndrome
- **POLG2** (published) mutation in a young female
- Homozygous **FTO** mutation in patient with clinical diagnosis of cerebro-oculo-facio-skeletal syndrome
- **FBN1** mutation causing Geleophysic dysplasia
- **C10ORF2** (TWINKLE) compound heterozygous mutations in Mitochondrial DNA Depletion syndrome 7
- **MCM4** compound heterozygous mutations in natural killer cell and glucocorticoid deficiency with DNA repair defect (NKGCD)
- **FRAS1** homozygous mutation in Fraser syndrome in a Product of Conception
- **RAB3GAP1** compound heterozygous mutations in Warburg Micro syndrome
- **COL4A1** mutation in muscle-brain-eye disease
- **KDM6A** mutation in Kabuki syndrome
- **ATP7A** hemizygous mutation in Menkes disease
- **SCN1A** mutation in Dravet syndrome
- **TH** homozygous mutation in tyrosine hydroxylase deficiency
- **TRMU** compound heterozygous mutations in acute liver failure in infancy with lactic acidemia
- Mosaic **CDKL5** mutation in a male with seizures, developmental delay, microcephaly, hypotonia
- **AP4E1** compound heterozygous mutations in Spastic paraplegia-51
- **MYL3** mutation in Hypertrophic cardiomyopathy
- Others: **JAG1, LMNA, WNT10A, CCM2, BRAF, KRAS, FOXP1, DPYD, PLA2G6, ATM, RAPSN, LICAM, MLC1, DNM2
- Many genes that were “candidates” have proven to be definitive soon after being reported
## ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Neoplasia, type 2</th>
<th>Loeys-Dietz Syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary Breast and Ovarian Cancer</td>
<td>Neurofibromatosis, type 2</td>
<td>Familial Thoracic Aortic Aneurysms and Dissections</td>
</tr>
<tr>
<td>Li-Fraumeni Syndrome</td>
<td>PTEN Hamartoma Tumor Syndrome</td>
<td></td>
</tr>
<tr>
<td>Peutz-Jeghers Syndrome</td>
<td>Retinoblastoma</td>
<td>Hypertrophic Cardiomyopathy</td>
</tr>
<tr>
<td>Lynch Syndrome</td>
<td>WT1-related Wilms Tumor</td>
<td>Dilated Cardiomyopathy</td>
</tr>
<tr>
<td>Familial adenomatous polyposis</td>
<td>Tuberous Sclerosis Complex</td>
<td>Catecholaminergic polymorphic ventricular tachycardia (CPVT)</td>
</tr>
<tr>
<td>MYH-Associated Polyposis; Adenomas, multiple colorectal, FAP type 2;</td>
<td>Hereditary Paraganglioma-Pheochromocytoma</td>
<td>Arrhythmogenic right ventricular cardiomyopathy (ARVC)</td>
</tr>
<tr>
<td>Colorectal adenomatous polyposis, autosomal recessive, with pilomatrixomas</td>
<td>Syndrome (PGL1, PGL2, PGL3, PGL4)</td>
<td></td>
</tr>
<tr>
<td>Von Hippel Lindau syndrome</td>
<td>EDS-vascular Type</td>
<td>Romano-Ward, Long QT Syndromes Types 1,2,3</td>
</tr>
<tr>
<td>Multiple Endocrine Neoplasia Type 1</td>
<td>Marfan Syndrome</td>
<td>Brugada Syndrome</td>
</tr>
<tr>
<td>Multiple Endocrine Neoplasia Type 2</td>
<td></td>
<td>Familiar Hypercholesterolemia</td>
</tr>
<tr>
<td>Familial Medullary Thyroid Cancer (FMTC)</td>
<td></td>
<td>Malignant Hyperthermia Susceptibility</td>
</tr>
</tbody>
</table>
Labs must report constitutional mutations found in the genes on the Minimum List ("ACMG56"), regardless of the indication for sequencing or the age of the patient.

Variants that have been previously reported AND are a recognized cause of the disorder

Or Variants that are previously unreported but are of the type that is expected to cause the disorder

Providers must provide comprehensive pre- and post-test counseling to the patient, including risks and benefits of sequencing and possibility of incidental findings that could require further evaluation.
In Practice….

- Initial analysis shows most individuals have multiple variants to be evaluated
  - Literature reviewed, etc.
- ACMG committee claims ~1% of individuals will have a reportable “incidental finding”
- Appears to be closer to 6-8%
- Sanger confirmation is not recommended in the guidelines, but still needed as false positive rates are a factor
- There is an ACMG maintenance committee discussing the issues and making new recommendations
### Making a Diagnosis in a Family

<table>
<thead>
<tr>
<th>Member</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proband</strong></td>
<td>Hearing impairment, cataracts at age 12y, kidney problems, absent reflexes, contractures, hypodontia and tooth agenesis.</td>
</tr>
<tr>
<td><strong>Mother</strong></td>
<td>No info provided, presumably normal/not evaluated</td>
</tr>
<tr>
<td><strong>Father</strong></td>
<td>No info provided, presumably normal/not evaluated</td>
</tr>
<tr>
<td><strong>Sister</strong></td>
<td>Progressive sensorineural hearing loss and early onset cataracts, kidney problems</td>
</tr>
</tbody>
</table>
## Two distinct genetic diagnoses in an individual

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Associated Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proband</strong></td>
<td>ABHD12 compound heterozygote (2 pathogenic variants)</td>
<td>autosomal recessive PHARC: polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataracts</td>
</tr>
<tr>
<td>Hearing impairment, cataracts at age 12y, kidney problems, absent reflexes, contractures,</td>
<td>WNT10A heterozygote</td>
<td>autosomal recessive form of ectodermal dysplasia; ALSO: &gt;50% of heterozygotes have features of ED, especially severe oligodontia (semi-dominant inheritance)</td>
</tr>
<tr>
<td>Hypodontia and tooth agenesis</td>
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Molecular Diagnosis + Clinical Picture

- **ABHD12**: p.D113fs, WT
  - **WNT10A**: WT, WT

- **ABHD12**: p.D113fs, p.V359fs
  - **WNT10A**: WT, p.F228I

- **ABHD12**: WT, p.V359fs
  - **WNT10A**: p.F228I, WT
Case from AJHG April 3, 2014

• 996 patients with moderate-severe intellectual disability
• Evaluated 565 candidate genes for ID
• 7 patients (0.7%) were found to have nonsense or frameshift mutations in the SETD5 gene
• SETD5 encodes a methyltransferase and is involved in histone modification
• SETD5 is in the critical region of the 3p25 microdeletion syndrome (with 2 other genes)
De novo dominant pathogenic variants in SETD5 gene in 7 ID patients
Clinical Features of Patients with SETD5 mutations

- Mod-severe ID
- Autism, OCD, hand-flapping
- Brachycephaly
- High forehead, synophrys/full eyebrows
- Long, thin, “tubular” nose
- Long, narrow palpebral fissures
- Large, fleshy, low-set ears
- Other – skeletal abnormalities, CHD, inguinal hernia, hypospadias, normal growth/HC and no seizures
Thank You!