Pediatric Genomic Medicine

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Children’s Mercy Hospital
Center for Pediatric Genomic Medicine

- Established Jan., 2011
  - Directed by Dr. Stephen Kingsmore

- Integrated with hospital practice
  - 25 physicians as primary points of contact representing every specialty within hospital
  - Clinical Genetics & Counseling
  - CMH Center for Bioethics

- Three application focuses
  - Exome sequencing
  - TaGSCAN CLIA lab test
  - STAT-seq Emergency Genome Sequencing
Single gene diseases are a proving ground for neonatal genomic medicine

- Variants are phenotypically deterministic
  - Disease $\equiv$ mutation(s)
  - Interpretable

- 20 year experience with gene sequencing for diagnosis
  - CLIA/CAP laboratory guidelines and certification
  - ACMG reporting conventions for variants
  - FACMG medical geneticist physicians
  - FACMG laboratory director interpretation
  - Genetic counselors
Diagnostic Complexity in the NICU

• Admissions to NICU are unexpected
  – 5% of US newborns, genetic testing potentially relevant for as many as 30% of admissions

• Diagnosis must be rapid to guide clinical decisions
  – Usual tests take 8 weeks

• Genetic diseases **look similar** in neonates
  – 3,757 known single gene diseases
  – Early in disease progression = incomplete symptoms, non-classical presentation
  – Phenotypic overlap: diseases have similar clinical features
  – Genetic heterogeneity: several genes cause same “disease”
  – Poorly defined clinical heterogeneity
24-hour Medical Genome Sequencing

Clock

0.0

- Identify newborn who may benefit
- Parental consent, DNA sample
Brother and sister stillborn at 27 weeks gestation

- Presented with a complex set of abnormalities
- Very extensive post-mortem work-up did not give a diagnosis
- After the first baby died, the parents were told the disease was “sporadic” = would not recur
- The parents wanted answers
- They want to have another child
24-hour Medical Genome Sequencing

Clock

0.0
- Identify newborn who may benefit
- Parental consent, DNA sample

3.0
- Sonication/ PCR-free Library Prep
- Clinical finding entry: SSAGA

Decreased from 1 day to 3 hours
Problem: Physicians don’t know which tests to order
Solution: SSAGA - Symptom and Sign Assisted Genome Analysis Software

SSAGA also:
- Accelerates interpretation
- Limits secondary (incidental) findings

Software limits regions of genome where variants reported
SSAGA Symptom and Sign Entry Page

**Search**

Select one or more clinical presentation terms and click Search to see genes and diseases associated with those terms.

Ctrl-click to select multiple terms in each category or to de-select terms.

### Clinical findings

#### Cardiovascular/Pulmonary
- cardiomyopathy
- chronic cough
- conduction disorder of heart
- congenital anomaly of lung

#### Constitutional/Metabolic
- Alpha-fetoprotein raised
- allergic state
- anorexia
- autoimmune state
- diffuse inflammation

#### Endo/GU/Renal
- advanced bone age
- ambiguous genitalia
- blood in urine
- congenital anomaly of the urinary system
- congenital malformation of genital organs

#### Gastrointestinal
- abdominal pain
- abnormal liver function
- achalasia
- anorectal anomaly
- bile duct proliferation

#### ID/Immuono/Heme
- allergic state
- anemia
- autoimmune state
- bacterial infectious disease
- bleeds easily

#### Neuro/Muscular/Development
- altered mental status / coma
- ataxia
- autistic disorder
- autonomic dysfunction
- cerebral calcifications

#### Derm
- alacrima
- alopecia
- angiokeratoma
- atrophic condition of skin
- blister of skin or mucosa

#### HEENT
- cherry red spot
- coarse features
- coloboma
- congenital anomaly of ear
- congenital anomaly of larynx

#### Skeletal/Growth
- advanced bone age
- arthritis
- bone pain
- congenital anomaly of hand
- congenital anomaly of skeletal bone

[Home Login (admin only)]
Brother and Sister’s Symptoms and Signs

<table>
<thead>
<tr>
<th>Cardiovascular/Pulmonary</th>
<th>Constitutional/Metabolic</th>
<th>Derm</th>
</tr>
</thead>
<tbody>
<tr>
<td>conduction disorder of heart</td>
<td>edema</td>
<td>hypopigmentation</td>
</tr>
<tr>
<td>congenital anomaly of lung</td>
<td>fever</td>
<td>keratosis</td>
</tr>
<tr>
<td>congenital heart disease</td>
<td>gout</td>
<td>nail finding</td>
</tr>
<tr>
<td>cyanosis</td>
<td>hydrops fetalis</td>
<td>papules</td>
</tr>
<tr>
<td>disorder of cardiac function</td>
<td>hyperammonemia</td>
<td>photosensitivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endo/GU/Renal</th>
<th>Gastrointestinal</th>
<th>HEENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>advanced bone age</td>
<td>abdominal pain</td>
<td>coloboma</td>
</tr>
<tr>
<td>ambiguous genitalia</td>
<td>abnormal liver function</td>
<td>congenital anomaly of ear</td>
</tr>
<tr>
<td>blood in urine</td>
<td>achalasia</td>
<td>congenital anomaly of larynx</td>
</tr>
<tr>
<td>congenital anomaly of the urinary system</td>
<td>anorectal anomaly</td>
<td>disorder of sclera</td>
</tr>
<tr>
<td>congenital malformation of genital organs</td>
<td>bile duct proliferation</td>
<td>dysmorphic facies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ID/Immuno/Heme</th>
<th>Neuro/Muscular/Development</th>
<th>Skeletal/Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>allergic state</td>
<td>muscle hypertrophy</td>
<td>pes cavus</td>
</tr>
<tr>
<td>anemia</td>
<td>muscle pain</td>
<td>short stature disorder</td>
</tr>
<tr>
<td>autoimmune state</td>
<td>muscle weakness</td>
<td>skull finding</td>
</tr>
<tr>
<td>bacterial infectious disease</td>
<td>occipital meningocele</td>
<td>talipes equinovarus</td>
</tr>
<tr>
<td>bleeds easily</td>
<td>predisposed to startle</td>
<td>tall stature</td>
</tr>
</tbody>
</table>

Home Login (admin only)
# Genes

**1,439 Genes Match the brother and sister’s Symptoms**

1,439 diseases found

searched for: congenital anomaly of lung, hydrops fetalis, nail finding, congenital anomaly of ear, dysmorphic facies, muscle weakness, talipes equinovarus

new search

<table>
<thead>
<tr>
<th>gene</th>
<th>search term(s)</th>
<th>disease(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAAS</td>
<td>muscle weakness</td>
<td>ACHALASIA-ADDISONIANISM-ALACRIMA SYNDROME</td>
</tr>
<tr>
<td>ABCA12</td>
<td>dysmorphic facies</td>
<td>ICHTHYOSIS CONGENITA, HARLEQUIN FETUS TYPE</td>
</tr>
<tr>
<td>ABCA3</td>
<td>congenital anomaly of lung</td>
<td>SURFACTANT METABOLISM DYSFUNCTION, PULMONARY, 3</td>
</tr>
<tr>
<td>ACAD9</td>
<td>muscle weakness</td>
<td>DEFICIENCY OF ACYL-CoA DEHYDROGENASE FAMILY MEMBER 9</td>
</tr>
<tr>
<td>ACADL</td>
<td>muscle weakness</td>
<td>ACYL-CoA DEHYDROGENASE, LONG-CHAIN, DEFICIENCY OF</td>
</tr>
<tr>
<td>ACADM</td>
<td>muscle weakness</td>
<td>ACYL-CoA DEHYDROGENASE, MEDIUM-CHAIN, DEFICIENCY OF</td>
</tr>
<tr>
<td>ACADVL</td>
<td>muscle weakness</td>
<td>ACYL-CoA DEHYDROGENASE, VERY LONG-CHAIN, DEFICIENCY OF</td>
</tr>
<tr>
<td>ADAMTSL2</td>
<td>dysmorphic facies</td>
<td>GELEOPHYSIC DYSPLASIA</td>
</tr>
<tr>
<td>ADCK3</td>
<td>muscle weakness</td>
<td>COENZYME Q10 DEFICIENCY</td>
</tr>
<tr>
<td>AGL</td>
<td>dysmorphic facies, muscle weakness</td>
<td>GLYCOCEN STORAGE DISEASE III</td>
</tr>
<tr>
<td>AHI1</td>
<td>dysmorphic facies</td>
<td>JOUBERT SYNDROME 3</td>
</tr>
<tr>
<td>ALG1</td>
<td>dysmorphic facies</td>
<td>CONGENITAL DISORDER OF GLYCOSYLATION, TYPE Ik</td>
</tr>
<tr>
<td>ALG12</td>
<td>dysmorphic facies</td>
<td>CONGENITAL DISORDER OF GLYCOSYLATION TYPE Ig</td>
</tr>
<tr>
<td>ALG2</td>
<td>muscle weakness</td>
<td>CONGENITAL DISORDER OF GLYCOSYLATION TYPE II</td>
</tr>
<tr>
<td>ALG3</td>
<td>dysmorphic facies</td>
<td>CONGENITAL DISORDER OF GLYCOSYLATION, TYPE Id; CDG1D</td>
</tr>
<tr>
<td>ALPL</td>
<td>dysmorphic facies</td>
<td>HYPOPHOSPHATASIA, CHILDHOOD</td>
</tr>
<tr>
<td>ALS2</td>
<td>muscle weakness</td>
<td>JUVENILE AMYOTROPHIC LATERAL SCLEROSIS 2</td>
</tr>
<tr>
<td>AMT</td>
<td>muscle weakness</td>
<td>GLYCINE ENCEPHALOPATHY</td>
</tr>
<tr>
<td>ANTXR2</td>
<td>dysmorphic facies</td>
<td>HYALINOSIS, INFANTILE SYSTEMIC</td>
</tr>
<tr>
<td>APTX</td>
<td>muscle weakness</td>
<td>ATAXIA, EARLY-ONSET, WITH oculomotor apraxia AND HYPOALBUMINEMIA</td>
</tr>
</tbody>
</table>
24-hour Medical Genome Sequencing

Clock

0.0
- Identify newborn who may benefit
- Parental consent, DNA sample

3.0
- Sonication/ PCR-free Library Prep
- Clinical finding entry: SSAGA

21.0
- HiSeq 2500 2x101 cycles x 140Gbp

Decreased from 11 days to 18 hours
18 hour 2 x 101 cycle sequence quality

**Q score distribution**

- **Omni**
  - 26,743
  - 2,296,369
- **WGS**
  - 43,882
  - 2.36M Omni5 snp chip locations
24-hour Medical Genome Sequencing

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21.0
• HiSeq 2500 2x101 cycles x 140Gbp

23.9
• Sequence alignment to reference, variant detection, genotyping: iSAAC
• Variant annotation: RUNES

Decreased from 24 hours to 3 hours
Finding Needles in a haystack = finding disease-causing variants in a genome

<table>
<thead>
<tr>
<th></th>
<th>MG12-1259</th>
<th>MG12-1259</th>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing time (hours)</td>
<td>26.5</td>
<td>25.5</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Aligned nucleotides</td>
<td>111 GB</td>
<td>128 GB</td>
<td>115 GB</td>
<td>112 GB</td>
</tr>
<tr>
<td>Depth of coverage</td>
<td>32.7</td>
<td>35.7</td>
<td>33.1</td>
<td>32.8</td>
</tr>
<tr>
<td>Total Nucleotide Variants</td>
<td>3,734,022</td>
<td>3,761,733</td>
<td>3,766,638</td>
<td>3,700,788</td>
</tr>
</tbody>
</table>
Standardized Variant Annotation by RUNES
(Rapid Understanding of Nucleotide variant Effect Software)

Each Variant → RUNES Standardized annotation → American College of Medical Genetics Pathogenicity Classification → Pathology Interpretation

- affected gene(s)/transcript(s)/ protein(s)
- NCBI Gene
- reference and variant codons
- reference and variant AA
- cDNA, CDS and AA position(s)
- SIFT
- POLYPHEN2
- BLOSUM
- dbSNP rsID
- dbSNP minor allele frequency
- HGMD cross reference
- Splicing effects
- OMIM cross reference
- Translation impact
- Frame shift
- CMH allele frequency
# American College of Medical Genetics variant pathogenicity categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Previously reported, recognized cause of the disorder</td>
<td>HGMD variation, dbSNP Snp CORE</td>
</tr>
<tr>
<td>2</td>
<td>Novel, of a type expected to cause the disorder</td>
<td>loss of initial premature stop codon, disruption of whole transcript, frameshifting, disruption of CDS/intron, overlap with non-synonymous in-frame insertion, disruption of overlap with</td>
</tr>
<tr>
<td>3</td>
<td>Novel, may or may not be causative</td>
<td></td>
</tr>
</tbody>
</table>
24-hour Medical Genome Sequencing

Clock

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21.0
• HiSeq 2500 2x101 cycles x 140Gbp

23.9
• Secondary analysis: iSAAC
• Tertiary analysis: RUNES

24.0
• VIKING-assisted interpretation
• Provisional report

Decreased from 1 month to 10 minutes
VIKING: Brother and Sister Genomes

Pulls up diseases and genes

1% allele frequency cutoff

VIKING
Variant Integration and Knowledge Interpretation in Genomes

- Integrates SSAGA and RUNES outputs
- Clusters variants on a gene-by-gene basis
- Dynamic filtering by interpreter
Sanger Confirmation of NEB Mutations

c.18786 C>G (p.Tyr4561X)
c.18981 C>G (p.Tyr6327X)

- Very unusual presentation of Nemaline Myopathy Type 2—would likely not have been chosen for Sanger sequencing
Benefits of rapid diagnosis of genetic diseases

- Gives an answer to the family: Ends uncertainty, guilt
- Genetic counseling of risk of recurrence
- ~500 genetic diseases have treatments
- Rules out diseases, avoiding unnecessary treatment
- Occupational therapy, physical therapy, special education, social work, life planning, support groups
Proof of Concept to Reality – April 2013

• Elevated maternal α-fetoprotein at 16 weeks gestation
• Fetal MRI: omphalocele, right hydronephrosis, large left hydrocele, curvature of thoracolumbar spine
• Delivered in CMH materno-fetal health center
• Admitted to NICU for treatment of giant ruptured omphalocele.
• Normal testing: Beckwith Wiedemann syndrome, karyotype and microarray
• At 2 months, elevated transaminases progressed to acute liver failure with an increased PT and PTT
Family consented 4/10; proband findings 4/13; father 4/18; mother 4/21

487 proband compound het PRF1 p.Ala437Val (rare, highly conserved, predicted to be damaging) and p.Ala91Val (8% MAF but pathogenic in presence of 2nd mutation)
488 mother: het p.Ala437Val
489 father: het p.Ala91Val
Criteria for clinical diagnosis of HLH

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fever</td>
<td>No</td>
</tr>
<tr>
<td>2. Hepatomegaly or splenomegaly</td>
<td>Modest splenomegaly</td>
</tr>
<tr>
<td>3. Cytopenia: hemoglobin&lt;9 g/dL, platelets &lt;100,000/mm³, ANC &lt;1000 (need 2)</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Serum ferritin &gt;500 ng/mL</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Serum triglyceride &gt;265 mg/dL or fibrinogen &lt;150 mg/mL</td>
<td>Yes</td>
</tr>
<tr>
<td>6. Absent/decreased natural killer cell assay</td>
<td>Yes, after Dx</td>
</tr>
<tr>
<td>7. Soluble IL2 receptor (CD25) &gt;2,400 units/mL</td>
<td>No</td>
</tr>
<tr>
<td>8. Hemophagocytosis without malignancy</td>
<td>Not done</td>
</tr>
</tbody>
</table>

- Confirmatory testing
  - NK activity: none
  - Perforin expression reduced

- Meds changed, steroids & IV Ig continued
16 of 326 Patients Had a Change in Diagnosis

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Prior gene tests</th>
<th>Old Diagnosis</th>
<th>New Diagnosis</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Change in Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joubert syndrome</td>
<td>2</td>
<td>AHI1 (het mutation; het VUS)</td>
<td><em>RPRIP1L</em></td>
<td>p.Asn1202IlefsX4</td>
<td>p.Lys1007X</td>
<td>Genetic counseling</td>
</tr>
<tr>
<td>Hypotonia, seizures, skin biopsy, elevated VLCFA</td>
<td>7</td>
<td>PEX nos</td>
<td><em>PEX5</em></td>
<td>p.Arg572Trp</td>
<td>p.Arg572Trp</td>
<td>Genetic counseling</td>
</tr>
<tr>
<td>Hydrocephalus, spasticity, encephalopathy, hypotonia</td>
<td>7</td>
<td>“mitochondrial”</td>
<td><em>PDHA1</em></td>
<td>p.Arg270X</td>
<td>n/a</td>
<td>Ketogenic diet</td>
</tr>
<tr>
<td>ID, behavior disorder, seizures</td>
<td>3</td>
<td>Carnitine deficiency</td>
<td><em>GAMT</em></td>
<td>c.327G&gt;A</td>
<td>c.299_311fs</td>
<td>Creatinine supplement</td>
</tr>
<tr>
<td>ID, seizures, dysmorphic, consanguinity</td>
<td>3</td>
<td>none</td>
<td><em>SNAP29</em></td>
<td>c.520+1G&gt;T</td>
<td>c.520+1G&gt;T</td>
<td></td>
</tr>
<tr>
<td>Cockayne syndrome / trichothiodystrophy</td>
<td>1</td>
<td>ERCC6; poss. deletion</td>
<td><em>ERCC2</em></td>
<td>p.Leu461Val</td>
<td>p.Asp655Tyr</td>
<td>Genetic counseling</td>
</tr>
<tr>
<td>Seizures, poor vision, behavior, consanguinity</td>
<td>1</td>
<td>CLN6 het</td>
<td><em>CLN8</em></td>
<td>p.Cys174Ser</td>
<td>p.Cys174Ser</td>
<td>Genetic counseling</td>
</tr>
<tr>
<td>Anemia</td>
<td>1</td>
<td>HBA1</td>
<td><em>HBB</em></td>
<td>p.Glu27Lys</td>
<td>n/a</td>
<td>Genetic counseling</td>
</tr>
<tr>
<td>Sanfilippo syndrome</td>
<td>none</td>
<td>none</td>
<td><em>SGSH</em></td>
<td>p.Arg150Gln</td>
<td>p.Ser66Leu</td>
<td></td>
</tr>
<tr>
<td>Congenital Myopathy; developmental delay; vent dependent</td>
<td>5</td>
<td>none</td>
<td><em>IGHMBP2</em></td>
<td>p.Leu361Pro</td>
<td>p.Arg71X</td>
<td></td>
</tr>
<tr>
<td>Congenital cardiomyopathy; death</td>
<td>1</td>
<td>LCHAD</td>
<td><em>VLCAD</em></td>
<td>p.Arg59Trp</td>
<td>p.Phe214Val</td>
<td>Low LCFA diet</td>
</tr>
<tr>
<td>Hypotonia, per. neuropathy, IUGR</td>
<td></td>
<td></td>
<td><em>IGHMBP2</em></td>
<td>p.Glu382Lys</td>
<td>p.Glu382Lys</td>
<td></td>
</tr>
</tbody>
</table>
## Medical Genome Sequencing

**Summary:**

- 15 affected children, 25 individuals, 11 families
- Definite diagnosis for 7 patients
- Likely diagnosis for 3 patients
  - novel disease gene in 2 sibs (*BCL9L*)
  - novel disease presentation (*GJB2*)
- 2 possible diagnoses: choanal atresia
- No diagnosis in 3
- 1 patient had change in treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>Description of illness</th>
<th>Causal Gene</th>
<th>Pattern of Inheritance</th>
<th>Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDT2</td>
<td>Tay Sachs Disease</td>
<td>HEXA</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>UDT173</td>
<td>Menkes disease</td>
<td>ATP7A</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH64</td>
<td>Erosive dermatitis</td>
<td>GJB2</td>
<td>De novo dominant</td>
<td>Y</td>
</tr>
<tr>
<td>CMH76</td>
<td>Mitochondrial disorder</td>
<td>?</td>
<td>?</td>
<td>N</td>
</tr>
<tr>
<td>CMH172</td>
<td>Neonatal epilepsy</td>
<td>BRAT1</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH184</td>
<td>Heterotaxy</td>
<td>BCL9L</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH185</td>
<td>Heterotaxy</td>
<td>BCL9L</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH186</td>
<td>Mother, Carrier</td>
<td>Carrier</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH202</td>
<td>Father, Carrier</td>
<td>Carrier</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH222</td>
<td>Choanal atresia</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>CMH223</td>
<td>Choanal atresia</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>CMH224</td>
<td>Mother, Unaffected</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>MG-12-12258</td>
<td>Lethal Multiple Pterygium syn.</td>
<td>NEB</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>MG-12-12259</td>
<td>Lethal Multiple Pterygium syn.</td>
<td>NEB</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH248</td>
<td>Mother, Carrier</td>
<td>Carrier</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH249</td>
<td>Father, Carrier</td>
<td>Carrier</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH488</td>
<td>Liver failure, omphalocele</td>
<td>PRF1</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH488</td>
<td>Mother, Carrier</td>
<td>Carrier</td>
<td>Recessive</td>
<td>Y</td>
</tr>
<tr>
<td>CMH489</td>
<td>Father, Carrier</td>
<td>Carrier</td>
<td>Recessive</td>
<td>Y</td>
</tr>
</tbody>
</table>
TaGSCAN - Targeted Gene Sequencing & Custom Analysis

- CLIA-lab test for 514 genes and 768 genetic diseases
  - Metabolic, mitochondrial, neurologic
  - Not arrhythmias, deafness

- All mutation harboring regions of genes

- Uses SSAGA to limit analysis to relevant disease genes
  - Decreased VUS, incidental findings, carrier status

- Cost $1000 - $3180, 3-8 week time-to-result
TaGSCAN Clinical Validation

- 326 archived CMH samples
  - Clinically sequenced with known mutations
  - Blinded TaGSCAN
  - 167 of 175 mutations detected (95%)
  - 99 of 110 samples with both pathogenic alleles (90%)

- 2 samples, compared with Omni5 SNP chip
  - Analytic sensitivity: 95%
  - Specificity: 99.8%

- Day-to-day precision: 99.3%
Two sisters with progressive cerebellar atrophy

<table>
<thead>
<tr>
<th></th>
<th>CMH000001</th>
<th>CMH000002</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>9 yrs</td>
<td>5 yrs</td>
</tr>
<tr>
<td><strong>Motor milestones</strong></td>
<td>normal</td>
<td>delayed</td>
</tr>
<tr>
<td><strong>Speech</strong></td>
<td>normal</td>
<td>delayed</td>
</tr>
<tr>
<td><strong>Ataxia</strong></td>
<td>4-5 yrs</td>
<td>2 yrs</td>
</tr>
<tr>
<td><strong>Loss of motor skills</strong></td>
<td>4-5 yrs</td>
<td>No</td>
</tr>
<tr>
<td><strong>Wheelchair reliance</strong></td>
<td>Yes, 8 yrs</td>
<td>No</td>
</tr>
<tr>
<td><strong>Progressive cerebellar atrophy</strong></td>
<td>Yes, 6 yrs</td>
<td>Yes, 4 yrs</td>
</tr>
</tbody>
</table>
Pyramid of perplexity

Pyruvate Decarboxylase Deficiency DNA testing $1600
GFAP gene sequencing for Alexander disease $1300
Array comparative genomic hybridization $1500
DNA testing for ataxia telangiectasia $1448
DNA testing for Freidreich’s ataxia $282
Lactic acid level: 4.3 elevated (x2) $90
Pyruvate: 0.23 elevated (x3) $1074
MELAS/MERRF DNA testing $864
Urine organic acids (x2) $1188
Karyotyping: 46,XX $517
Acylcarnitine profile $134
Ammonia (plasma) $23
Urine amino acids $267
Brain MRI x2 $7784
Vitamin E level $170
Brain MRS $4204
TSH, free T4 $74
Copper $149
AFP $177
BMP $13
LFTs $9
CBC $7

5 year diagnostic work-up: $23,000, no molecular diagnosis
Diagnosis: Ataxia with oculomotor apraxia, type 1

Both have homozygous mutations that create a premature stop codon in *aprataxin*

The parents were unaffected carriers

### Actionable genomics

- *Aprataxin* mutations cause CoQ$_{10}$ deficiency

- 19¢ CoQ10 pill daily

- After 4 months
  - report better stamina
  - but worse by rating scale

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Secondary. Patients with all three forms of CoQ$_{10}$ deficiency have shown clinical improvements after initiating oral CoQ$_{10}$ supplementation. Thus, early diagnosis is of critical importance in the management of these patients.

**Muscle coenzyme Q10 deficiencies in ataxia with oculomotor apraxia 1**

Continuing Development

• Analytic sensitivity trumps diagnostic specificity
  – False negative variant calls more problematic than false positives

• Improved methods for detecting nucleotide variation
  – large in/dels > 40 nt,
  – variants in genes with pseudogenes,
  – triplet repeat expansions, CNVs, inversions etc.

• Optimal patient selection criteria

• Diagnostic design optimization
  – Affected WGS, 1st parent WGS during proband analysis, 2nd if needed