NGS Case Presentation - Genetics Point of View

Identification of the X-linked Inhibitor of Apoptosis Protein mutation using WGS

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Department of Pediatrics
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Medical College of Wisconsin
The following relationship(s) exist related to this presentation:

CHW/MCW offers fee for service genetic counseling and whole genome sequencing.
Presenter holds a specific patent related to the tertiary analysis of WGS data
Learning objectives

After this presentation, you should be able to discuss and explain:

• NGS WGS technologies including assay limitations, reference data issues and limitations, and analysis limitations
• Clinical WGS implementation, including existing tools for data analysis and interpretation
• Clinical indications for NGS testing including the use of confirmatory testing, functional analysis of variants, and issues surrounding delivery of results to clinicians
• The infrastructure required to support clinical WGS using the example of the Pediatric WGS Program at MCW/CHW
Request for help June 27th 2009

- Male who presented at ~1.5 years of age with poor weight gain and a perianal abscess
- Symptoms progressed rapidly over a few months to a very unusual early onset, aggressive, refractory inflammatory bowel disease
- In spite of aggressive therapy:
  - Immunosuppressants
  - Diverting colostomy at 2 years
  - Total colectomy and partial ileostomy at 4 years
  - Complete bowel rest at 4.5 years
- Disease slows but continues to progress
- Many diagnostic tests were performed but none returned a diagnosis
What to do next?

- Several forms of immune dysfunction have been associated with inflammatory bowel disease

- May respond to immune reconstitution or require alternate treatment plan dependent on the underlying cause

- Clinical question: Could we use next generation sequencing to establish if we should carry out a transplant?
  - No transplant will be carried out without a diagnosis

- We can try!
Methodology

• Selection of sequencing methodology
  – Exome sequencing (cost decision)
    • Nimblegen capture
  – Roche 454 sequencing (in house already and availability of donated runs)
    • Requires 5 runs to derive an average coverage of at least 35x

• Selection of analysis path to obtain variant list
  – Vendor tools for primary and secondary analysis

• In-house tool development for tertiary analysis (to identify candidate mutations)
  – Pipelines but also manual steps e.g. analysis of sequence reads to identify possible sequencing/mapping errors

• Expert interpretation of candidate mutations

• Sanger confirmation of candidates by external CLIA diagnostic lab

• Additional follow up assays as required
CarpeNovo – A variant analysis tool for clinical use

More than a dozen variant file input formats accepted

12 reference databases supplying up to ~60 data genomic annotations to the variant, transcript, or gene

5 additional reference sources supplying ~30 additional annotations

Provides close to 100 computed annotations - annotated to the variant, transcript, or gene

College of American Pathologists regulatory approval obtained
Variant filtering/prioritisation

By Pre-existing Targeting: Gene, Gene Set, Region, or All Genes

Exclude Likely Errors: Quality Score, Allele Support, Pseudogene etc.

By Novelty or Expected Allele Frequency: TGP, dbSNP, ESP, Valcrie

By Importance based on Conservation: PhastCons, PhyloP

By Expected Effect on Protein: PolyPhen, SIFT, Splice, Premature Stop etc.

By Anticipated Mode of Inheritance: Zygosity, Pseudoautosomal

By Genic Location: Protein Coding/Non-Synonymous/Promoter

By Existing Phenotype Association: HGMD/OMIM

By Presence in Internal Control Datasets
Additional annotations displayed in feature level reports

Search Results in Summary:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant #</th>
<th>Genetist Processed</th>
<th>Analyst Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCC11</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ABCC8</td>
<td>2</td>
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<tr>
<td>ADORA3</td>
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<td>0</td>
</tr>
<tr>
<td>AGT</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Entrez GeneID: 6833  
GeneName: ATP-binding cassette, sub-family C (CFTR/MRP), member 8
ProteinIDs: Q09428, Q59GMS

Description: The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MRP subfamily which is involved in multi-drug resistance. This protein functions as a modulator of ATP-sensitive potassium channels and insulin release. Mutations and deficiencies in this protein have been observed in patients with hyperinsulinemic hypoglycemia of infancy, an autosomal recessive disorder of unregulated and high insulin secretion. Mutations have also been associated with non-insulin-dependent diabetes mellitus type II, an autosomal dominant disease of defective insulin secretion. Alternative splicing of this gene has been observed; however, the transcript variants have not been fully described. [provided by RefSeq]

Position: 11:17371009-17455025

OMIM Association:  
- Diabetes mellitus, noninsulin-dependent, 125853 (3)  
- Diabetes mellitus, permanent neonatal, 606176 (3)  
- Diabetes mellitus, transient neonatal 2, 610374 (3)  
- Hyperinsulinemic hypoglycemia, familial 1, 256450 (3)  
- Hypoglycemia of infancy, leucine-sensitive, 240800 (3)

Mutations: Show by Mutation Types, Show by Diseases

<table>
<thead>
<tr>
<th>Type</th>
<th>Disease</th>
<th>m,RN &gt; VN</th>
<th>p,RA &gt; VA</th>
<th>Variant Class</th>
<th>Source</th>
<th>Pub Link</th>
<th>Gene Card</th>
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</thead>
<tbody>
<tr>
<td>Splicing</td>
<td>Hyperinsulinism</td>
<td></td>
<td></td>
<td>Disease-causing</td>
<td>Biobase HGMD</td>
<td>11395395</td>
<td>abcc8</td>
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<tr>
<td>Missense/Nonsyns</td>
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<td>p.C&gt;R435</td>
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<tr>
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<tr>
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</table>
### Variant Details

#### Conservation

<table>
<thead>
<tr>
<th>Nuc Ref</th>
<th>Pos</th>
<th>Score</th>
<th>Rating</th>
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<tr>
<td>T</td>
<td>17431351</td>
<td>1</td>
<td>High sequence conservation</td>
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#### Transcripts

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<thead>
<tr>
<th>Transcript_ID: NM_000352</th>
<th>Variant Location: EXON</th>
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#### Amino Acid Predictions:

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<tr>
<th>Ref</th>
<th>Var</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y: neutral, polar, cyclic, aromatic, large</td>
<td>C: neutral, nonpolar - hydrophobic, acyclic, small, tiny</td>
<td>nonsynonymous</td>
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</table>

#### Potential Splice Site Altering Variant: F

<table>
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<tr>
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<td>sequence ami</td>
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<tr>
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<td>numObserv</td>
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<td>84</td>
<td>Protein ID</td>
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<tr>
<td>Q09428</td>
<td>PDB ID</td>
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#### SIFT Prediction

<table>
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<th>Protein</th>
<th>Orthologue Prediction</th>
<th>Orthologue Score</th>
<th>Homologue Prediction</th>
<th>Homologue Score</th>
<th>Amino Acid Change</th>
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</thead>
<tbody>
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<td>NP_000343</td>
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<td>TOLERATED</td>
<td>1</td>
<td>Y356C</td>
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#### Known HGMD or Alternate Source Mutation

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y356C</td>
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</tbody>
</table>
CarpeNovo software was used to identify the mutation causing the disease.

- Input variants from exome sequencing
- Protein coding or possible splice site altering
- Non-synonymous
- Novel (versus dbSNP129)
- Homozygous or hemizygous
- Likely to be damaging
- Altering highly conserved nucleotides
- Absent in other reference genomes

* GSTM1 transferase – many null mutations known
The gene:

XIAP

X-LINKED INHIBITOR OF APOPTOSIS; XIAP

BACULOVIRAL IAP REPEAT-CONTAINING PROTEIN 4; BIRC4

Gene map locus Xq25

- Known to be a critical regulator of apoptosis - the process of coordinated, programmed cell death that occurs in multicellular organisms.

- Important in: termination e.g. of damaged, infected, or aberrantly performing cells; homeostasis - maintaining balance in cell numbers; tissue development; and lymphocyte interactions – appropriate development of the immune system.
The variant changes the second cysteine in the BIR2 domain (C203) to a tyrosine (Y203)

This region of the protein is responsible for mediating interactions with many other proteins

This cysteine is critical for both the structural integrity and the function of this region; it is a highly conserved amino acid

Image from C. Sun et al 1999
Phylogenetic conservation

All obtainable sequenced individuals within all of these species have a cysteine. At this time it was more than 5,000 chromosomes, currently it is ?
Newly identified role in the inflammatory response

In addition to its defined role in apoptosis, it was becoming clear that XIAP is at the center of the proinflammatory cytokine response to pathogens.
Clinical diagnostic confirmation

Sequencing findings confirmed by external CLIA lab (using Sanger sequencing)

The clinical lab reported the variant as a mutation in the sample

The child’s mother was found to be heterozygous for the variant

X-inactivation studies showed that there is abnormal X-inactivation in the mother
Mutation would be predicted to affect apoptosis

Mutation would be predicted to affect release of inflammatory molecules
Follow-up studies

Analysis of these pathways allows formation of testable hypotheses

Apoptosis
- Research test developed and carried out in CHW CLIA lab
- The child’s cells are more sensitive to apoptosis (measured by CD3 stimulation of PBMC blasts)
- Significantly more cell death compared with controls

Inflammatory response
- Research test developed and carried out in CHW CLIA lab
- The child’s cells do not respond to bacterial stimuli (Tri-DAP and MDP) by producing inflammatory molecules (IL-8)
- The child’s pro-inflammatory signaling pathway is defective

Reduced XIAP expression also seen (but possibly an effect of the altered structure of the protein)

In binding assays the mutant XIAP shows significantly decreased RIP2 binding
The diagnosis led to a change in treatment

- Based on these findings and molecular diagnostic and immunological follow up the child was diagnosed with a functional defect in the XIAP protein.
- This defect caused an immune dysregulation syndrome resulting in an early onset, severe, and unusual IBD.
- This diagnosis guided selection of a treatment – a hematopoietic cord blood transplant.
- The child received the transplant (7 months after final diagnosis; following second opinions and recovery from setback).
- He recovered well, and was able to eat (from day 42). We believe that the treatment ultimately saved the child’s life.
- He recently celebrated his 7th birthday, is attending school and has had no recurrence of the GI or other aspects of the disease.
Not the end of the story; The MCW Pediatric WGS MDx Program

**Goal:** Clinical utilization for diagnostic purposes in a pediatric population
- Not research; not for creation of generalizable knowledge

**Purpose:** Define molecular etiology of diseases for medical decision-making
- End a diagnostic odyssey
- Actionable

**Process:** Carefully oversight and management
- 3 phases
- 4-6 cases reviewed monthly
- Case review
- Careful consent
- Sequencing to variant calling carried out externally
- Analysis/interpretation carried out at MCW
- Follow up counseling
Disclosure decisions

• Parents are always informed if sequencing reveals a pediatric disease with a defined treatment regardless of whether $1^0$ or $2^0$
• We allow parents to decide whether they want to learn about diseases that fall into other categories:

<table>
<thead>
<tr>
<th>Mandatory disclosures</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1^o$ diagnostic</td>
</tr>
<tr>
<td>$2^o$ treatable childhood</td>
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<table>
<thead>
<tr>
<th>Optional disclosures</th>
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<tbody>
<tr>
<td>$2^o$ none</td>
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<tr>
<td>$2^o$ not actionable childhood</td>
</tr>
<tr>
<td>$2^o$ actionable adulthood</td>
</tr>
<tr>
<td>$2^o$ not actionable adulthood</td>
</tr>
</tbody>
</table>

• Only those variants confirmed by a second method in line with patient/parental preferences become part of the EHR
Examples

**Childhood onset medically actionable**
- Biotinidase deficiency
  - Unable to recycle the vitamin biotin
  - Seizure, hypotonia, ataxia, developmental delay
  - Biotin Rx prevents all problems

**Childhood onset medically not actionable**
- Tay-Sachs disease
  - Hexosaminidase A deficiency
  - Unable to degrade glycosphingolipid GM2 ganglioside in the brain
  - Progressive neurodegeneration; death by 4

**Adult onset medically actionable**
- BRCA1 – autosomal dominant cancer
  - Common before 50 yo
  - 57% breast by 70 yo 40% ovarian by 70 yo
  - Can has mastectomy & oophorectomy
  - Reduces risk 90%

**Adult onset medically not actionable**
- Familial Alzheimer – autosomal dominant
  - PSEN1, PSEN2, APP
  - Onset in 40’s & 50’s. Severe memory failure eventually incapacitating
  - Confusion, poor judgment, language disturbance, agitation, hallucinations
Counseling Time

- Exploration of expectations: 16%
- Inheritance: 14%
- Test Methodology: 9%
- Categorical Model of Choice: 18%
- Psychosocial Counseling: 19%
- Follow-up Planning: 10%
- Formal consent: 8%
- Clinical genetics evaluation: 9%
- 6-10 hours total
Patient decisions to date

60 cases have gone before the committee
18 have been approved
14 have moved beyond counseling

<table>
<thead>
<tr>
<th>Proceed with WGS</th>
<th>None*</th>
<th>Not actionable Childhood</th>
<th>Actionable Adult</th>
<th>Not actionable Adult</th>
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<tbody>
<tr>
<td>Yes</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>% Yes</td>
<td>92.3</td>
<td>8.3</td>
<td>83.3</td>
<td>58.3</td>
</tr>
</tbody>
</table>

* Other than mandatory pathogenic and actionable childhood onset disease
Insurance Companies are willing, in certain circumstances, to pay for WGS 4 out of 10 pre-authorizations

February 23, 2011

David Dimmock, M.D., Associate Professor
David Bick, M.D., Associate Professor
Division of Genetics, Dept of Pediatrics
Medical College of Wisconsin
HRC, Rm. H5865
8701 Watertown Plank Road
Milwaukee, WI 53226

We are interested in the development of an evaluation clinic where you will assess our insured children and adults before expensive diagnostic testing is performed and evaluate the likely expected costs of routine testing. As we have discussed, in the situations where you determine that on average the costs of routine testing will exceed the current contract price of whole genome sequencing we will authorize whole genome sequencing as the first line clinical test.

We are committed to continuing to establish the utility of whole genome sequencing beyond these currently agreed indications, and are excited about the ongoing clinical utility monitoring you have established as part of your clinical whole genome sequencing program.
Additional tools developed to support WGS MDx

CarpeNovo
Variant annotation
Reference data
Variant querying
Analyst reports
Cross sample analyses
Clinical reports

Valcrie
Reference genome variants
Reference genome phenotype data
Reference genome individual and sample metadata

GapMine
Average coverage calculations
Overcoverage
What's not covered - gap analysis
Low coverage
Clinical reports

GATool
Functional annotation
Data from multiple species
Ontology driven
Curated pathway data
External links

DiseasePortal
Manually curated disease and phenotype data
Ontology driven
Ontology based enrichment calculations
Targeted by disease focus

Text Mining
PubMed mining
Ontology based
Mining for gene/phenotype/disease terms
Prioritisation by selection of genes with little existing info in DBs

Expert analysis and interpretation
You can’t identify the mutation if you didn’t sequence the region

- It is important to know which regions **have not** been sequenced
  - When attempting to exclude regions of interest; for example a particular gene family where members are already associated with the phenotype
  - Lack of sufficient depth of coverage may result in uncertain zygosity calls; this has to be defined and reported

---

<table>
<thead>
<tr>
<th>Average depth of coverage</th>
<th>Gap Report</th>
<th>Genes with gaps</th>
<th>Nature of gaps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undercovered regions</td>
<td>Number of gaps in genome</td>
<td>% gene in gaps</td>
<td>Low complexity</td>
</tr>
<tr>
<td>Overcovered regions</td>
<td>% genome in gaps</td>
<td>% transcripts in gaps</td>
<td>Repeats</td>
</tr>
<tr>
<td></td>
<td>% exons in gaps</td>
<td>Reference genome gap</td>
<td></td>
</tr>
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</table>

Identification of sequence gaps and coverage information
Sequencing gaps in known disease genes

Two unrelated individuals share this particular gap; one has developmental delay the other is immunodeficient; it is also found in other samples.

We see gaps in the exonic proportion of many other disease associated genes that are shared across the genomes of individuals with diverse phenotypes (as well as being seen in our healthy control samples)
This sequence coverage information ends up in the medical report

“All exons of all genes were analyzed for depth of coverage. This analysis includes the genes in table 1. Those genes in table 1 with insufficient depth of coverage to be fully analyzed are listed in table 2. Because of insufficient depth of coverage, the genes in table 2 may harbor variants that were not detected by this test. Depth of coverage of introns is not documented in this table.”

These findings can direct strategies for additional testing
For example whether single gene re-sequencing should be performed
We need tools to determine whether a candidate gene has an interesting function. GATool for integrated functional annotation

Annotation Search and Export

Step One: Define a list of Genes to annotate

Select a Species

Enter Gene Symbols

Enter a genomic region (Optional)

To do this clinically we need to have control over the data; this use case limits simple use of external tools that are subject to change outwith our control.
Selection of annotations

Step Two: Select annotations to include in report
Functional annotations

RGD based gene summary; driven by gene, disease, phenotype, and pathway ontologies; indicates associations based on structured data

RefSeq based gene summary

OMIM based gene - disease summary

HGMD based gene – disease summary

EntrezGene based gene summary

UniProt based gene summary

GeneCards based gene summary

GeneWiki based gene summary

HugeNavigator gene summary
### Phenotypic annotations

#### Section 3: Known Phenotypes Related to Gene Summary

<table>
<thead>
<tr>
<th>Gene Symbol:</th>
<th>TBX2A2R</th>
<th>Mouse Ortholog:</th>
<th>Tbx2a2r</th>
<th>Rat Ortholog:</th>
<th>Tbx2a2r</th>
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<tbody>
<tr>
<td>RGD ID:</td>
<td>735834</td>
<td>Ortholog RGD ID:</td>
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<td>Species: Rat</td>
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<td>Link to Gene Report</td>
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<td>Link to Gene Page</td>
<td>RGD</td>
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</table>

#### Ontologies:
- GO
- Disease
- Pathway
- Mammalian Phenotype

#### Annotations

<table>
<thead>
<tr>
<th>Disease</th>
<th>Specific terms</th>
<th>Evidence</th>
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#### GO: Cellular Component

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Clinical Report

- Indication for test
  - Phenotype and patient details

- Diagnosis
  - Details of the mutations identified

- Interpretation
  - Explanation of the likely impact of the mutation

- Recommendation
  - Genetic counseling, potentially follow up testing for particular mutations

- Method
  - Wet bench and computational methodology

- Limitations
  - Wet bench and computational - including regions not sequenced

- References
  - General and specific to diagnosis
Take home messages

• WGS MDx can be used to make a diagnosis today!
• We haven’t and won’t always find the mutation:
  – Patients /families made aware of this possibility from the onset
  – Sometimes the gene-disease associations will come after the fact
  – The success rate across institutes is ~1:20 (MCW higher)
• We will find secondary results:
  – We should not blind ourselves to these
• We will find important pharmacogenomics data:
  – Especially likely in patients who have been very ill for a long time
• We won’t always (will rarely) have a pedigree or cohort (or even parents) for comparison:
  – Rare diseases are sometimes too rare
  – In specified timeframe there is no time to find additional patients
More take home messages

• Diseases will be redefined:
  – Disease will eventually be defined (and treated) by the variant (or the protein domain or network) not the collection of symptoms
  – This will revolutionize the practice of medicine, improving diagnostics and helping physicians optimize care for their patients

• May lead to knowledge that no suitable treatments exist:
  – Testing may lead to decision to provide palliative care
  – Patients and families must be made aware of this from the onset

• WGS approach will affect drug selections:
  – in some cases increasing and in some cases decreasing options

• Education of all types of providers (primary care and specialists) will be necessary:
  – Providers will need a working knowledge of genomics
Why are we doing this?

- Initially presented with poor feeding and failure to thrive, but met early developmental milestones
- Subsequently began to fail to meet milestones then developed seizures
- Many tests performed but nothing abnormal identified
- At 3.5 years further testing identified an intracellular Cobalamin (vitamin B12) metabolism disorder
- She was started on a treatment
- Progression of her disease stopped but the neurologic abnormalities already present likely not reversible
- The mutation is likely to have been found via WGS; it is believed that early intervention with the same treatment would have resulted in normal neurological development
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