MOLECULAR PATHOLOGY CASE STUDIES
- CONNECTING THE DOTS BETWEEN GENETICS, MOLECULAR BIOLOGY & BIOCHEMISTRY IN REAL PATIENTS -

WHAT YOU NEED TO BRING!
- A good understanding of genetics, molecular biology & biochemistry...review the last 2½ weeks of sessions!
- An internet-enabled device (laptop, tablet, etc.).
- Your thinking cap!

With recent advances in the integration of various disciplines of molecular science and technological developments in genetic analysis, it is now possible to implement truly “personalized” medicine. The growing adoption of “Precision Medicine” involves the full understanding of a patient, including their own specific molecular pathology and disease etiology, which can help to establish an accurate diagnosis and to select an effective therapy.

NCBI has long had online resources for biologists to explore what is known about a biological molecule including its structure and function, but has recently developed clinically-focused resources enabling scientists and clinicians to integrate known molecular biological information with clinically-relevant genetic variations.

In Thursday’s Session:
- We will discuss the state of clinical practice with regard to the application of precision medicine principles.
- Together we will explore a real-world case study and follow a workflow to discover the patients’ molecular pathology for an undiagnosed/misdiagnosed problem.
- You will then be given a practice case study to solve, and we will go over the case and how to present your findings in preparation for.....

Before Friday’s Session:
- Your group will be assigned your own case study to explore and discover what is happening in your patient on the molecular level. You will be guided to prepare a summary that you can share with the class.

In Friday’s Session:
- Each groups’ case will be presented to the class so that you can see additional examples of molecular pathology in real patients - and see diversity of molecular pathology even in patients with the same disease or who have pathogenic genetic variants in the same gene.

Facilitator: Rana Morris, PhD - an NCBI Customer Experience team member and Team Lead for Educational Programs (Courses/Workshops, Webinars, Educational Materials). Since 2002, she has provided user support and training, as well as working with supervisors and development teams to improve NCBI resources based on user-centered design principles. Her doctoral, post-doctoral and research fellowship work integrated disciplines of computational and experimental biochemistry, molecular and cellular biology and genetics, and has included diagnostic development, drug design and coordination of genetics/genomics components of clinical trials.
Foundations of Medicine: FDN164 & FDN167

Case Session:
Case-based Genetics, Molecular Biology and Biochemistry
Integrated Review

August 30, 10am-12pm and August 31, 1:30-3:30pm
(both sessions meet in Ross 101)
What’s going to happen today & tomorrow?

In Today’s Session:
• We will discuss the state of clinical practice with regard to the application of “Precision Medicine” principles (examining a patient’s specific molecular pathology).
• Together we will explore a real-world case study and follow a workflow to discover the patients’ molecular pathology for an undiagnosed/misdiagnosed problem.
• You will then be given a practice case study to solve, and we will go over the case and how to present your findings in preparation for.....

Before Friday’s Session (after Today’s session):
• Your Group will be assigned your own case study to explore and discover what is happening in your patient on the molecular level. You will be guided to prepare a summary that you can share with the class.

In Friday’s Session:
• Each Groups’ case will be presented to the class so that you can see additional examples of molecular pathology in real patients - and see diversity of molecular pathology even in patients with the same disease or who have pathogenic genetic variants in the same gene.

Session Objectives

FDN164 (Part 1.....Thursday)
1. Recognize molecular biology and genetic factors that are relevant to underlying disease processes
2. Identify and apply on-line information databases toward explanation of molecular biology and genetics of disease processes
3. Discuss the dysfunctional molecular biology and genetics associated with a diagnosed disease
4. Apply principles of basic sciences to clinical scenarios

FDN167 (Part 2.....Friday)
1. Recognize significant details in patient’s case relevant to underlying disease processes
2. Identify any additional information required and where/how to get it
3. Present details pertinent to their specific case and discover / discuss important factors in related cases down to the molecular level
4. Apply principles of basic sciences to studying clinical scenarios
Let’s get this out of the way – “How am I going to be tested on this?”

You are going to be tested on this in several ways:

Next week’s exam
- You will not have to run through a whole case study.
- You will not be tested on the specifics of each of these cases.
- You will need to be able to answer questions based on application of the cases:
  - when genetics is something to consider and how it fits within clinical cases
  - why it helpful to integrate molecular sciences for decisions about patient care & case management
  - how problems at the molecular level (integrated molecular pathology) relate to health
  - where to find biological information when you need it!

USMLE Step 1
- integration of all aspects of molecular science will help you answer case study-driven questions

Your CAREER!
- Keeping up with science, technology with an increasingly-specific focus on individual patient’s cases
- Resident Preceptors, Attending Physicians, etc. – will all be asking you questions.....
- Patients & Parents wanting to know “why?”
THE COMING OF AGE - MOLECULAR MEDICINE

• The Art of Medicine

• The Science of Medicine

• Evidence-based Clinical Practice
  "Evidence-based medicine is the integration of best research evidence with clinical expertise and patient values."

• “Personalized” Medicine
  [AMA – all patient-care should be “personalized”]

• Precision (including Genomic) Medicine
  “Precision medicine is an emerging approach for disease diagnosis treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.”

• and now? ..... 

YET ANOTHER NEW TERM: HIGH-DEFINITION MEDICINE

Establish an Individual’s Baseline of Health
Create a Personalized “Prevention” Strategy
Perform High-Definition Diagnosis & Select High-Precision Treatment

“Precision” Medicine - Disease Characterization for Research, Diagnosis & Therapeutic Selection

When to think about “Clinical” Genetic Testing?

- Patients with a known genetic disease/disorder & a family history
- Patients with a known genetic disease/disorder & no family history
- Asymptomatic patients with a family history of a genetic disease/disorder

What to Take into Consideration….

Pros

- Decreasing in cost & not particularly invasive
- Known genetic lesion can sometimes help in drug or therapy selection
- Predict disorders even before symptoms begin – for proactive & preventative care.

Cons

- We are early in our understanding of genes, gene variants and disease.
  - Failure to detect a pathogenic variant does not rule out the diagnosis
  - Prediction isn’t guaranteed just yet - not all pathogenic variants cause the same symptoms in every patient (penetrance, severity, multi-genic & environmental influences)
- Lack of coverage by some insurance companies
**COMPANION DIAGNOSTICS & TARGETED THERAPIES**

**Acute Myeloid Leukemia (AML) - M2 subtype**
\[ t(6;9)(p23;q34) \]

**Chronic Myelogenous Leukemia (CML)**
\[ t(9;22)(q34;q11.2) \]

- **DEK-NUP214**
- **ABL1**
- **NUP214**

Survival rate early 1970s = <20%
Survival rate now (still) <20%

Survival rate early 1970s = <15%
Survival rate now >94%!

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**PHARMACOGENOMICS EXAMPLE: DOSAGE PREDICTION**

Dosing for Warfarin/Coumadin can be complicated....

**Example:**

- Young, african american, physically fit, male
  - high dose....120 mg
- Older, caucasian, smoking, slightly heavy, non-smoking female
  - low dose....2 mg
WHAT IS “NCBI”? 

We are a “center” within the NLM responsible for creation, curation and maintenance of medical and scientific databases and other things...

We receive, create, archive & make available biomedical information, as well as perform computational biology & IT systems research.....

but we really aspire to help make sense of the information!

NCBI’S INFORMATION HUBS & SOME DATABASES
PRESENTING SYMPTOMS:
developmental delay, dystonic movements, tremors, muscle hypotonia, unsteady gait, vomiting, drooling, sleep disturbances

STRANGE FINDINGS
conditions deteriorated and were temporal (significantly worse after 11am daily)

CONTINUED ISSUES AFTER
“SUCCESSFUL TREATMENT”
tremors, drooling, sleep disturbances, and eventually unexplained respiratory issues in Alexis
Might this be genetic? Here’s the family pedigree

*Late discovery, an unrelated neurological disorder runs in the maternal line of this family unit.

[Image of a family pedigree diagram]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3314311/

Review of family pedigree & genetic variation data

Variations found
SPR Gene: R150G & K251X

[Image of a graphic modified to highlight key points]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3314311/
So here’s where your case begins… and where you will begin all of your other cases.

Stepping through free, online government databases to integrate and understand what you know about patients & their biology!

[Diagram showing MedGen to Gene to RefSeq Gene to RefSeq Protein to Conserved Domains (CDD) to Structure]

MyClinicalService

Physician Referral Form

Patient Information

Patient Name: ALEXIS & NOAH

DOB, Medical Record Number (MRN):

Requesting Provider

Assigned Provider/Practice Name: Jane Ferreiro, MD / MyClinicalService

Specialty/Department: Pediatrics/Neurology

Address: 900 23rd St NW, Washington, DC 20037

Phone: (202) 555-1212

Facsimile #: (202) 555-1212

Consultant Provider

Provider’s Name: to be assigned

Specialty/Department: Molecular Science/M1 Training

Address: 2300 I St NW, Suite 201, Washington, DC 20052

Phone: (202) 555-1212

Facsimile #: (202) 555-1212

Referral Information

Authorization No: Authorization Type:

Reason for Referral: Evaluation of Sepiapterin Reductase Deficiency

Diagnosis: G24.1 – Sepiapterin Reductase Deficiency

Clinical Notes: 12 year old twin boy and girl, initially diagnosed at 24 months with Cerebral Palsy due to chief complaints of hypotonia, dystonia and spasticity and the results of a brain imaging study on the male, then diagnosed at 5 years old with Dopa-responsive Dystonia (Sagawa Disease) and treated fairly successfully with L-Dopa. However, symptoms (sleep disturbances, mood disorders, drooling) were still present and at 14 years old, Alexis developed serious respiratory complications. A preliminary diagnosis of Sepiapterin Reductase Deficiency Syndrome was made based on normal levels of Neopterin and very low levels of BH4 and both Serotonin (5HIAA) and Dopamine (HVA) metabolites.

Whole genome sequencing was performed at the request of the family and will be analyzed for pathogenic variants that exist in both twins. The final report will be faxed to the Molecular Science/M1 Training program for evaluation.

Please consult with the family and send a copy of the final report back to this office. Thanks.

Procedures: Variant Interpretation – Molecular Impact Characterization

Visits Allowed: 3

Unit Type: V (VISIT)

Referral is Valid Until: 09/30/2018

Notes: Patient must arrive 30 minutes early, with a picture ID, Insurance card and have a copy of this referral. If the referred patient is a minor and anyone other than the child’s parents are escorting the child to the appointment, a letter of consent by the parent is needed. Please bring a list of medications the patient is taking with you to this appointment (including over the counter).

Please send the final report by Fax to: (202) 555-1212

Signature:

Ferreiro, Jane, MD on 08/29/2018 at 8:26 AM EDT
**Clinical test results for Dystonia Comprehensive Panel**

<table>
<thead>
<tr>
<th>GENE</th>
<th>RESULTS</th>
<th>EXPLANATION</th>
</tr>
</thead>
</table>
| **SPR (2p13.2)** | Pathogenic variant detected: Arg150 Arg150Gly | **This result supports the diagnosis of Sepiapterin reductase deficiency.** This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., 5HIAA, 5HVA, BH4, Neopterin, etc.).
A sequencing study with PCR validation has identified one copy of this reported pathogenic variation:
The Arg150Gly variation is an A to G change at nucleotide position 6075 in the SPR gene. This encodes an alternate residue at position 150 from a large, positively-charged, polar amino acid to one with a small, neutral side chain. |
| **SPR (2p13.2)** | Pathogenic variants detected: Lys251 Lys251Ter | **This result supports the diagnosis of Sepiapterin reductase deficiency.** This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., 5HIAA, 5HVA, BH4, Neopterin, etc.).
A sequencing study with PCR validation has identified one copy of this reported pathogenic variation:
Lys251Ter (SPR: g.9120A>T, c.751A>T, p.Lys251Ter) variation.
The Lys251Ter variation is an A to T change at nucleotide position 9120 in the SPR gene. This forms a premature stop codon at amino acid position 251 resulting in an abnormally short or truncated protein. |
| No genetic variants were detected in: | ANO3 (11p14.3-14.2) ATP1A3 (19q13.2) CIZ1 (9q34.11) DRD2 (11q23.2) GCH1 (14q22.2) GNAL (18p11.21) HPCA (1p35.1) KCTD17 (22q12.3) PNKD (2q35) PRKRA (2q31.2) PRRT2 (16p11.2) SGCE (7q21.3) SLC2A1 (1p34.2) SLC6A3 (5p15.33) TH (11p15.5) THAP1 (8p11.21) TOR1A (9q34.11) TOR1AIP1 (1q25.2) TUBB4A (19p13.3) | |
DISCLAIMER:
Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing should be considered.

CLINICAL DESCRIPTION
Dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive movements and/or postures. Dystonic movements are typically patterned and twisting, and may be associated with tremor. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation. Dystonia can be classified clinically according to age of onset, body distribution, temporal pattern, and associated features (i.e., isolated dystonia – in which it is the only motor feature except tremor; combined dystonia – in which another movement disorder is present; or complex dystonia – in which other neurologic or systemic manifestations are present).

Conditions tested:

<table>
<thead>
<tr>
<th>CONDITION(S)/PHENOTYPE(S)</th>
<th>ALSO KNOWN AS</th>
<th>GENE(S) TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystonia</td>
<td></td>
<td>All listed below and: HPCA (1p35.1) KCTD17 (22q12.3) PARK2 (6q26) PNKD (2q35) SLC2A1 (1p34.2) TOR1AIP1 (1q25.2)</td>
</tr>
<tr>
<td>Autosomal dominant torsion dystonia 4</td>
<td></td>
<td>TUBB4A (19p13.3)</td>
</tr>
<tr>
<td>Dystonia 1</td>
<td>Dystonia 1, modifier of Early-Onset Primary Dystonia (DYT1)</td>
<td>TOR1A (9q34.11)</td>
</tr>
<tr>
<td>Dystonia 10</td>
<td>EPISODIC KINESIGENIC DYSKINESIA 1, Familial Paroxysmal Kinesigenic Dyskinesia</td>
<td>PRRT2 (16p11.2)</td>
</tr>
<tr>
<td>Dystonia 12</td>
<td>Rapid-Onset Dystonia-Parkinsonism</td>
<td>ATP1A3 (19q13.2)</td>
</tr>
<tr>
<td>Dystonia 16</td>
<td></td>
<td>PRKRA (2q31.2)</td>
</tr>
<tr>
<td>Dystonia 23</td>
<td></td>
<td>CIZ1 (9q34.11)</td>
</tr>
<tr>
<td>Dystonia 24</td>
<td></td>
<td>ANO3 (11p14.3-14.2)</td>
</tr>
<tr>
<td>Dystonia 25</td>
<td></td>
<td>GNAL (18p11.21)</td>
</tr>
<tr>
<td>Dystonia 5, Dopa-responsive type</td>
<td>DYSTONIA, DOPA-RESPONSIVE, GTP Cyclohydrolase 1-Deficient Dopa-Responsive Dystonia</td>
<td>GCH1 (14q22.2)</td>
</tr>
<tr>
<td>Dystonia 6, torsion</td>
<td></td>
<td>THAP1 (8p11.21)</td>
</tr>
<tr>
<td>Infantile Parkinsonism-dystonia</td>
<td>DOPAMINE TRANSPORTER DEFICIENCY SYNDROME</td>
<td>SLC6A3 (5p15.33)</td>
</tr>
<tr>
<td>Myoclonic dystonia</td>
<td>DYSTONIA 11, MYOCLONIC</td>
<td>DRD2 (11q23.2) SGCE (7q21.3)</td>
</tr>
<tr>
<td>Segawa syndrome, autosomal recessive</td>
<td>Tyrosine Hydroxylase Deficiency, Tyrosine Hydroxylase-Deficient Dopa-Responsive Dystonia</td>
<td>TH (11p15.5)</td>
</tr>
<tr>
<td>Sepiapterin reductase deficiency</td>
<td>Dopa-Responsive Dystonia Due to Sepiapterin Reductase Deficiency</td>
<td>SPR (2p13.2)</td>
</tr>
</tbody>
</table>

METHODOLOGY
Full gene sequencing and deletion/duplication analysis of targeted gene coding regions are performed using Next-Generation (NGS)/Massively Parallel Sequencing (MPS). All pathogenic variants and deletions/duplications are confirmed using orthogonal technologies.

PERFORMANCE
Our analytic validation study has demonstrated >99.9% sensitivity and specificity for tested mutations.
Researching the Referral

1. To learn more about the preliminary diagnosis, go to the NCBI website (https://www.ncbi.nlm.nih.gov or “google” NCBI to find the homepage) and search NCBI’s MedGen database with: **sepiapterin reductase deficiency**

Understanding the Genetic Test Results

2. **What are the specific gene and variations identified in the twins?**
   *(Read the results, sometimes it is really helpful!)*

   **What does the genetic test result mean for their diagnoses?**

   You can find out what various genetic testing laboratories, clinical genetic organizations, and OMIM are claiming with regard to health-related impact for these genetic variations in the **ClinVar database**.

   You can search with a Gene Symbol and nucleotide or protein change, an rsID or an HGVS expression, for example type:

   **SPR Arg150Gly OR SPR Lys251Ter**
INFORMATION ABOUT THIS GENE FROM HUMAN-CURATED SOURCES:

3. On the MedGen record, **click the link for the gene** identified as having variants in the twins. **WHAT DOES THIS GENE NORMALLY DO?** (summary from NCBI’s RefSeq Group Curators)

4. From the Gene record, **scroll down to the General gene information>Gene Ontology section** to learn more about the protein produced from this gene. This section displays terms for where this gene product is likely to be found within a cell (Component), what processes it is often involved in (Process), and what it does (Function). **(terms assigned by the Gene Ontology Consortia’s Curators)**

**WHAT TYPE(S) OF PROCESS(ES) IS/ARE THIS PROTEIN NORMALLY INVOLVED WITH?**

**DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?**

**WHAT SPECIFIC FUNCTION(S) DOES THIS PROTEIN HAVE?**

*(Binding to ligands, substrates and/or cofactors; General and/or specific functional activities.)*

**DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?**

**IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?**

5. Now find the **Expression section** to see in which tissues this gene is expressed and, since the protein is maintained within the cell, where it functions.

**IN WHICH TISSUES HAS THIS GENE BEEN FOUND TO BE EXPRESSED?**

**DO ANY OF THESE CORRELATE WITH SOME OF THE TWINS’ SYMPTOMS?**

Based on your understanding of the complexity of gene expression, how might you **EXPLAIN SOME OF THE DIFFERENCES IN SYMPTOMS OBSERVED IN THE TWINS?**
INFORMATION ABOUT THIS GENE DETERMINED FROM SEQUENCE-BASED SOURCES:

6. From the Gene record, (on the right-hand side of the page) **click the “RefSeqGene” link** to see the “Graphic” view of the gene structure defined on the chromosome on a RefSeqGene nucleotide page.

There are several different “Tracks” available in this view, with names shown on the left. (You can manipulate what is shown by the “Tracks” button in the upper-right.)

At the very top of this view, the grey bar shows the region corresponding to the genome with a “ruler” above showing nucleotide or base/residue.

Below this is the **Genes** track with the following:

A green bar with the gene symbol (SPR) shown is the full length of the gene region with little arrow-heads indicating the 5' to 3' direction of the coding. There is a single “green” gene region for each gene annotated on the genome.

Underneath the **green bar** may be shown the gene products of the gene:

- The **purple bar** with the accession indicates regions that are transcribed into RNAs. For mRNAs, thicker regions are the designated “exons” and the connecting lines (with directional arrow-heads) representing the connecting “introns” (which will be removed by splicing) and the **red bar** indicates the regions on the mRNA exons that encode the protein (also called coding sequence or CDS).

For some genes, it is known that there are multiple transcript splice-variants formed due to RNA processing (splicing), these would be shown as addition purple (and corresponding red – if protein coding) tracks. In order to help to establish a common numbering system of exons (since there may be different combinations in different transcript variants), the **black bar** below shows numbered boxes corresponding to each possible exon identified for the particular gene.

WHERE IS/ARE THE TWINS’ GENETIC VARIANTS LOCATED IN THIS GENE AND IN THE mRNA?  
(on the picture above or on your screen – draw or visualize a vertical line at the position of each if the variants)

BASED ON THE POSITION(S) OF THE VARIANT(S) IN THE GENE, WHAT IS THE MOST LIKELY MECHANISM FOR IMPACTING THE FINAL GENE PRODUCT? (alter gene expression, influence transcript processing, or change encoded protein sequence)
7. On the RefSeqGene page, (on the right-hand side) you can click the “Protein” link or go back to the Gene record and **click the “RefSeq Proteins” link. Click “Graphics”** to see a graphical view of the annotated regions curated on the protein sequence. The information shown in these “tracks” of this view can help you to learn more about this protein.

WHERE IN THE PROTEIN SEQUENCE IS/ARE THE TWINS’ GENETIC VARIANTS LOCATED?
(on the picture above or on your screen – draw or visualize a vertical line at the position of each if the variants)

Take a look at the annotations shown in the Graphic view. **BASED ON WHERE IT THE VARIANT(S) IS/ARE LOCATED, WHICH MIGHT THE VARIANT(S) ALTER:**

- □ the protein’s **location** (signal peptide)
- □ post-translational **processing** of the protein (cleavage site)
- □ post-translational **modification** of the protein (phosphorylation or methylation site, for example)
- □ the **functional activity** of the protein (domain, motif, and/or specific site/“key” residue – binding, active site, catalysis, for example)

To learn more about the main functional regions of the protein **click “Identify Conserved Domains”**.

**WHAT MIGHT BE THE IMPACT OF THE GENETIC VARIATIONS ON THE PROTEIN’S FUNCTION?**

- There are several different “Tracks” available in this view, with names shown on the left. (You can manipulate what is shown by the “Tracks” button in the upper-right.)
  - At the very top of this view, the grey bar shows the length of the protein with a “ruler” above showing amino acid position/residue from N-terminus to C-terminus.
  - The “Protein Features” bar mimics the grey bar, above, and displays the gene name.
  - Other “Features” tracks indicate important residues for the function of the protein.
    - Mouse-over the features to learn more about each -
      - The “region Features - CDD” track shows bars corresponding to functional domains that have been annotated in this protein sequence by the NCBI Conserved Domain Curation Staff.
      - “region Features” indicates critical amino acid positions involved in the activity of the protein.
      - “site Features – CDD” shows specific amino acid positions that are critical for various functions of this protein (including binding, catalysis, active site, interface & protein-protein interactions, etc.)
      - “site Features” identifies other important amino acid positions which are predicted to be targets of other cellular processes (including sites for targeting with post-translational modifications or targets for proteolytic cleavage).
      - When there is evidence of Other tracks that are shown here when there is information to support it, include “signal peptide” if the protein is to be targeted to a specific subcellular location other than the cytosol.
8. From either the Gene or Protein record, you can click a link to 3D Structure to visualize experimentally-determined molecular structures for this protein. In the 3D structure you can see precisely the locations of the amino acids affected by the genetic variations.

To make things easier for you right now.....

here's a picture of the 3D crystal structure monomer of the Human SPR protein complex (PDB accession: 4Z3K) as displayed in NCBI's Cn3D Viewer.

The protein backbone is showed in a long red tube, with two bound substrates (NADPH & a sepiapterin analog) for the SPR reaction shown as ball-and-stick and spacefill rendering, respectively.

The position of where the variants would exist (Arg150 & Lys251) are highlighted in yellow and two additional important residues displayed in grey (Asp144) and white (Asp257).

**WHAT DO YOU THINK THE CHANGE IN AMINO ACIDS MIGHT DO TO THE 3D STRUCTURE AND FUNCTION OF THE PROTEIN?**

**Arg150** normally forms a salt-bridge with Asp144 to stabilize the protein’s 3D structure.

Loss of this salt-bridge, due to replacement of the negatively-charged arginine with a neutral glycine (Arg150Gly), causes the enzyme to unfold and lose all activity.

**The C-terminal end of the protein and Asp257**, in particular, stabilizes the binding and locks the sepiapterin substrate into the active site of the enzyme.

Loss of the C-terminal tail and this particular residue (as in the truncation variant Lys251Ter) causes loss of the ability of the enzyme to efficiently bind and lock-in its substrate – resulting in the loss of all activity.
**REVIEW OF MOLECULAR PATHOLOGY**

What we’ve found so far:

- Alexis & Noah have Sepiapterin reductase deficiency with double heterozygous pathogenic variants in the SPR gene.

- SPR is the catalyst of the rate-limiting step for the production of a cofactor Tetrahydropterin (BH₄) which is critical for the synthesis of neurotransmitters & nitric oxide.

- SPR is expressed in tissues such as brain, muscle, vasculature, connective tissue, stomach, salivary gland, adrenal gland and pineal gland, among others.

- The two pathogenic variants cause the production of very low or non-functional Sepiapterin reductase.
  - SPR:p.R150G *(from the father’s side)*: Disrupts the formation of a wild-type salt-bridge causing the unfolding of the enzyme’s 3D structure and targeting the protein for degradation.
  - SPR:p.K251X *(from the mother’s side)*: Causes the loss of the C-terminal tail including a D257, a critical binding residue for capturing, holding onto and coordinating the enzyme’s substrate for catalysis.

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**WHAT WAS WRONG WITH THE TWINS AND HOW WAS THIS USED TO “FIX” THEIR PROBLEMS?**
THE POINT OF DOING ALL OF THIS...

THE WORKFLOW THAT WE’VE USED IN THIS CASE STUDY WILL ALSO WORK FOR OTHER WELL-STUDIED AS WELL AS MANY POORLY-UNDERSTOOD DISORDERS.

• **GENE** is a “gene-related” information hub providing information and links to more for just about anything that is currently known about:
  • Sequence, expression, structure & function of the genes and gene products
  • Links to specific, relevant literature
  • Links to sources of research reagents & experimental studies with data

• **MEDGEN** is a “health-related” information hub, providing links to:
  • Information about diseases/conditions
  • Links to scientific/clinical publications & patient education materials
  • Links to the Genetic Testing Registry & ClinicalTrials.gov
  • Links to information about relevant disease-associated genes
NOW, IT’S YOUR TURN!

TASKS TO DO & QUESTIONS TO ANSWER

• Work in your group or in “groups” (2s, 3s, 4s)
• Follow the worksheet....
• Be prepared to answer the questions at the end.
  (If you get stuck – keep trying.....but if you really get stuck
  – raise your hand and I’ll come around to help unstick you.)

• We’ll get back together to go over this....
• If you hear the music playing, you’ve got about 2min to finish up.
### Requesting Provider

**Assigned Provider/Practice Name:**
Jane Ferreiro, MD / MyClinicalService  
**Address:**
900 23rd St NW  
Washington, DC 20037  
**Phone:** (202) 555-1212  
**Facsimile #:** (202) 555-1212

### Consultant Provider

**Provider’s Name:**
to be assigned  
**Address:**
2300 I St NW, Suite 201  
Washington, DC 20052  
**Phone:** (202) 555-1212  
**Facsimile #:** (202) 555-1212

### Referral Information

**Reason for Referral:** Evaluation of Marfan Syndrome (Q87.4)

**Diagnosis:** Death due to Thoracic aortic dissection

**Clinical Notes:** A 35 year old, caucasian male was found unresponsive on his kitchen floor and pronounced dead on the scene by EMS, who delivered the body to the Medical Examiner’s Office. Tox screen was negative with no evidence of trauma. Cause of death was determined to be thoracic aortic dissection.

The cause of death as well as other typical clinical features present in the body that are commonly associated with Marfan Syndrome (tall and lanky stature, long and narrow face with deeply set eyes, and pectus excavatum) concerned the family who requested additional investigative measures.

Follow up on a full diagnosis is important because the patient was reported to have visited two local emergency rooms in the three days prior to death with apparent mis-diagnoses of food poisoning and generalized viral syndrome, respectively.

A blood sample has been sent out for Marfan Syndrome - targeted variant analysis (genetic testing). The genetic test result report will be faxed to the Molecular Science/M1 Training program for evaluation.

Please consult with the family and send a copy of the final report back to this office. Thanks.

**Procedures:** Variant Interpretation – Molecular Impact Characterization

<table>
<thead>
<tr>
<th>Visits Allowed</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Type</td>
<td>V (VISIT)</td>
</tr>
<tr>
<td>Referral is Valid Until</td>
<td>09/30/2018</td>
</tr>
<tr>
<td>Notes</td>
<td>A representative of the family must arrive 30 minutes early, with a picture ID, Insurance card and have a copy of this referral.</td>
</tr>
</tbody>
</table>

Please send the final report by Fax to: (202) 555-1212

**Signature:**

Ferreiro, Jane, MD on 08/29/2018 at 10:15 AM EDT
Clinical test results for Marfan/TAAD

9 conditions tested:

- Thoracic aortic aneurysm and aortic dissection (TAAD)
- Arterial tortuosity syndrome (ATS)
- Congenital contractural arachnodactyly (CCA)
- Ehlers-Danlos syndrome (EDS)
- Homocystinuria
- Loeys-Dietz syndrome (LDS)
- Marfan syndrome (MFS)
- Shprintzen-Goldberg syndrome (SGS)
- X-linked mental retardation with marfanoid habitus syndrome

<table>
<thead>
<tr>
<th>GENE</th>
<th>TEST RESULTS</th>
<th>EXPLANATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBN1</td>
<td>Arg1790 Arg1790Ter</td>
<td>This result confirms the diagnosis of or predisposition for Marfan Syndrome (MFS). This result should be interpreted in the context of clinical presentation and results of other laboratory tests. A PCR/sequencing study has confirmed one copy of the Arg1790Ter (FBN1: g.194098C&gt;T, c.5368C&gt;T or p.Arg1790Ter) variation. The Arg1790Ter mutation is caused by a C to T change at nucleotide position 194098 in the FBN1 gene and results in a change from an arginine to a stop (termination) codon at position 1790. This causes a premature termination of translation and produces an abnormally shortened protein. This individual's result has important implications for other family members. Clinical and laboratory evaluations should be considered for at risk individuals. Genetic counseling is recommended for at risk individuals.</td>
</tr>
<tr>
<td>ACTA2 CBS COL3A1 COL5A1 COL5A2 FBN2 FLNA MED12</td>
<td>MYH11 SKI SLC2A10 SLC2A SMAD3 TGFB2 TGFB1 TGFR1</td>
<td>Negative</td>
</tr>
</tbody>
</table>
DISCLAIMER:
Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing should be considered.

ASSAY METHODS
Full-Gene Sequencing covers the full gene coding sequence, +/- 10 base pairs of adjacent intronic sequence, and other non-coding sequence positions containing select known pathogenic variants. Deletion/Duplication Analysis detects most intragenic deletions and duplications at single exon resolution. Rarely however, single-exon duplication events may be missed due to inherent sequence properties or isolated reduction in data quality.

CLINICAL DESCRIPTION
Familial thoracic aortic aneurysm and dissection (familial TAAD) involves problems with the aorta, which is the large blood vessel that distributes blood from the heart to the rest of the body. Familial TAAD affects the upper part of the aorta, near the heart. This part of the aorta is called the thoracic aorta because it is located in the chest (thorax). Other vessels that carry blood from the heart to the rest of the body (arteries) can also be affected. In familial TAAD, the aorta can become weakened and stretched (aortic dilatation), which can lead to a bulge in the blood vessel wall (an aneurysm). Aortic dilatation may also lead to a sudden tearing of the layers in the aorta wall (aortic dissection), allowing blood to flow abnormally between the layers. These aortic abnormalities are potentially life-threatening because they can decrease blood flow to other parts of the body such as the brain or other vital organs, or cause the aorta to break open (rupture). The occurrence and timing of these aortic abnormalities vary, even within the same affected family. They can begin in childhood or not occur until late in life. Aortic dilatation is generally the first feature of familial TAAD to develop, although in some affected individuals dissection occurs with little or no aortic dilatation. Aortic aneurysms usually have no symptoms. However, depending on the size, growth rate, and location of these abnormalities, they can cause pain in the jaw, neck, chest, or back; swelling in the arms, neck, or head; difficulty or painful swallowing; hoarseness; shortness of breath; wheezing; or coughing up blood. Aortic dissections usually cause severe, sudden chest or back pain, and may also result in unusually pale skin (pallor), a very faint pulse, numbness or tingling (paresthesias) in one or more limbs, or paralysis. Familial TAAD may not be associated with other signs and symptoms. However, some individuals in affected families show mild features of related conditions called Marfan syndrome or Loeys-Dietz syndrome. These features include tall stature, stretch marks on the skin, an unusually large range of joint movement (joint hypermobility), and either a sunken or protruding chest. Occasionally, people with familial TAAD develop aneurysms in the brain or in the section of the aorta located in the abdomen (abdominal aorta). Some people with familial TAAD have heart abnormalities that are present from birth (congenital). Affected individuals may also have a soft out-pouching in the lower abdomen (inguinal hernia), an abnormal curvature of the spine (scoliosis), or a purplish skin discoloration (livedo reticularis) caused by abnormalities in the tiny blood vessels of the skin (dermal capillaries). However, these conditions are also common in the general population. Depending on the genetic cause of familial TAAD in particular families, they may have an increased risk of developing blockages in smaller arteries, which can lead to heart attack and stroke.

-from GHR
Researching the Referral

1. To learn more about the preliminary diagnosis, go to the NCBI website (https://www.ncbi.nlm.nih.gov or “google” NCBI to find the homepage) and search NCBI’s MedGen database with: marfan syndrome

Understanding the Genetic Test Results

2. WHAT ARE THE SPECIFIC GENE AND VARIATIONS IDENTIFIED IN JONATHAN? (Read the results, sometimes it is really helpful!)

WHAT DOES THE GENETIC TEST RESULT MEAN FOR JONATHAN’S DIAGNOSIS?

Molecular Biology Research

INFORMATION ABOUT THIS GENE FROM HUMAN-CURATED SOURCES:

3. On the MedGen record, click the link for the gene identified in Jonathan on the genetic test result. WHAT DOES THIS GENE NORMALLY DO? (summary from NCBI’s RefSeq Group Curators)

You can find out what various genetic testing laboratories, clinical genetic organizations, and OMIM are claiming with regard to health-related impact for these genetic variations in the ClinVar database.

You can search with a Gene Symbol and nucleotide or protein change, an rsID or an HGVS expression, for example type: FBN1 p.Arg1790Ter
4. From the Gene record, scroll down to the General gene information>Gene Ontology section to learn more about the protein produced from this gene. This section displays terms for where this gene product is likely to be found within a cell (Component), what processes it is often involved in (Process), and what it does (Function). (terms assigned by the Gene Ontology Consortia’s Curators)

**WHAT TYPE(S) OF PROCESS(ES) IS/ARE THIS PROTEIN NORMALLY INVOLVED WITH?**
**DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?**

**WHAT SPECIFIC FUNCTION(S) DOES THIS PROTEIN HAVE?**
(Binding to ligands, substrates and/or cofactors; General and/or specific functional activities.)
**DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?**

**IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?**

**INFORMATION ABOUT THIS GENE DETERMINED FROM SEQUENCE-BASED SOURCES:**

5. From the Gene record, (on the right-hand side of the page) click the “RefSeqGene” link to see the “Graphic” view of the gene structure defined on the chromosome on a RefSeqGene nucleotide page.

![Gene Structure Image](image)

**WHERE IS JONATHAN’S GENETIC VARIANT LOCATED IN THIS GENE AND IN THE mRNA?**
(On the picture above or on your screen – draw or visualize a vertical line at the variant’s position.
You can type in the variant’s gene position, from the genetic test result, into the “Find” box to automatically zoom in!)

**BASED ON THE POSITION OF THE VARIANT IN THE GENE, WHAT IS THE MOST LIKELY MECHANISM FOR IMPACTING THE FINAL GENE PRODUCT?**
(alter gene expression, influence transcript processing, or change encoded protein sequence)
6. On the RefSeqGene page, (on the right-hand side) you can click the “Protein” link or go back to the Gene record and click the “RefSeq Proteins” link. Click “Graphics” to see a graphical view of the annotated regions curated on the protein sequence. The information shown in these “tracks” of this view can help you to learn more about this protein.

WHERE IN THE PROTEIN SEQUENCE IS JONATHAN’S GENETIC VARIANT LOCATED?
(On the picture above or on your screen – draw or visualize a vertical line at the variant’s position. You can type in the variant’s protein position, from the genetic test result, into the “Find” box to automatically zoom in!)

WHAT MIGHT BE THE IMPACT OF THE GENETIC VARIATION ON THE PROTEIN’S STRUCTURE & FUNCTION?

7. Structurally, Fibrillin-1 (green) aggregates with Fibronectin (red) and other proteins (including Elastin) in long chain meshes of structurally-reinforcing microfibrils in connective tissue (see schematic on the left). This plays a major role in arteriole tunica media (the pink portion of the cross-section schematic on the right) to strengthen the vasculature and is particularly needed in regions susceptible to stress (ex: aorta).

WHAT DO YOU THINK COULD BE THE FUNCTIONAL IMPACT OF THE INCORPORATION OF JONATHAN’S TRUNCATED FORM OF THE PROTEIN INTO THIS COMPLEX?
Introduce your patient!

“*I’ll take care of this one!*”

What was the preliminary diagnosis & the rationale?
(check the referral #1)

What did the genetic test find and how does this relate to the preliminary diagnosis?
(check the genetic test result #2)

What is the implicated gene and what is its normal function?
(see #3 and #4)

Where in the gene and gene product(s) is the genetic variant located?
(see #5)

What is the molecular impact of the genetic variant on the gene product?
(see #6)

What *do you think might be* the functional impact of the variant on the gene product?
(see #7)
Self-Assessment:
*Take a minute to write down your thoughts….*

**My initial ideas about the case:**
- Why did I think this?
- How confident was I?

**What did I miss?**
- Why did I miss it?
- How could I have thought about it differently?
You’ll get your own Group Case to work on!

What now?

Today or tomorrow morning.....
• Get together with your Group and work together on your assigned case.
• Come up with a summary that your group can share tomorrow.
  *There’s a guide-sheet in the back that you can fill out.*
• Don’t spend more than an hour on this!

Tomorrow, we’ll share each of your cases with the class and discuss them.
• We’ll share those cases so that everyone can see how molecular mechanisms impact real people’s disease/conditions.
• I will ask Groups to answer those specific summary questions.
  *I will ask for volunteers first....but if no one volunteers, I’ll start picking on groups.*
  *It’s okay if you don’t exactly get-it....I’ll help out!*
• We’ll look at all of these cases in context and discuss major concepts that they demonstrate – which may be good exam topics.
NCBI Workshops | References & Resources

THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION, a.k.a. THE “NCBI”  

ABOUT THE NCBI

NCBI NEWS & SOCIAL MEDIA:
   Facebook: https://www.facebook.com/ncbi.nlm
   Twitter: https://twitter.com/NCBI
   YouTube Channel: https://www.youtube.com/user/NCBINLM
   LinkedIn: https://www.linkedin.com/company/3595640

HelpDesk EMail Address: info@ncbi.nlm.nih.gov

RESOURCES MENTIONED IN TODAY’S PRESENTATION

CLINICAL RESEARCH

MedGen – Aggregated information about medical genetic conditions and phenotypes  

NLM’s ClinicalTrials.gov – A registry and results database for clinical studies 
   Homepage: http://clinicaltrials.gov

Genetic Testing Registry (GTR) – NIH’s registry of genetic tests and laboratories  

ClinVar – A collection of assertions about the relationships of genomic variations with human health  

U.Washington’s GeneReviews – A resource of clinically relevant and medically actionable information for inherited conditions  
   Homepage: https://www.ncbi.nlm.nih.gov/books/NBK1116/

NLM’s Genetics Home Reference – Consumer-friendly information about genetic variations and health  

JHU’s OMIM – A compendium of human genes and genetic phenotypes  

NLM’s Medline Plus – Consumer information about diseases, conditions, and wellness issues  
   Homepage: http://www.nlm.nih.gov/medlineplus/

MOLECULAR ETIOLOGY RESEARCH

Gene – Aggregated information with links to genomic, expression, homolog, structure and function data  

RefSeq – A project to create a comprehensive, integrated, non-redundant, well-annotated reference set of annotated sequences for genomes, chromosomes, transcripts and proteins.  
   RefSeq Project Homepage: https://www.ncbi.nlm.nih.gov/refseq/  
   RefSeqGene Homepage: https://www.ncbi.nlm.nih.gov/refseq/rsg/  

Conserved Domains Database (CDD) – A database of protein domain information with sequence fingerprints  

Structure – A database of 3D macromolecular structures and complexes from the Protein Database (PDB)  