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 Wednesday’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 has been assigned this 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.

Ashanti

Here’s the patient’s referral and the genetic test results for the molecular pathology work up.

Needs to be done and ready for presentation by Friday!

Thanks
## Patient Information

**Patient Name**: ASHANTI

**DOB, Medical Record Number (MRN)**: 

## Requesting Provider

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

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

**Specialty/Department**: Pediatrics/Allergy & Immunology

**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

**Specialty/Department**: Molecular Science/M1 Training

**Phone**: (202) 555-1212

**Facsimile #:** (202) 555-1212

## Referral Information

**Reason for Referral**: Evaluation of Severe Combined Immunodeficiency

**Diagnosis**: D81.3 – Severe combined immunodeficiency [SCID] due to Adenosine Deaminase Deficiency

**Clinical Notes**: 4 year old girl was diagnosed with SCIDs after 2 years of repeated infections and over 11 blood transfusions. She was then placed in sterile-isolation in the hospital or at home. A new enzyme assay identified an adenosine deaminase deficiency which lead to treatment with regular injections of recombinant PEG-ADA. However, a new gene therapy trial has just begun with a goal of permanently replacing the gene for the affected enzyme. Confirmation testing to validate the diagnosis of SCID due to a pathogenic variant in Adenosine Deaminase is required for qualifying for the procedure.

A blood sample has been sent out for analysis with a SCIDs genetic testing panel. 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

**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 4:15 PM EDT
Clinical test results for Severe Combined Immunodeficiency (SCID)

<table>
<thead>
<tr>
<th>GENE</th>
<th>TEST RESULTS</th>
<th>EXPLANATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA (20q13.12)</td>
<td>Gly216Arg</td>
<td>This result confirms the diagnosis of Severe Combined Immunodeficiency (SCID) due to Adenosine Deaminase Deficiency. This result should be interpreted in the context of clinical presentation and results of other laboratory tests. A PCR/sequencing study has confirmed two copies of the Gly216Arg (ADA: g.33697G&gt;A, c.646G&gt;A or p.Gly216Arg) variation. The Gly216Arg mutation is caused by a G to A change at nucleotide position 646 in the ADA gene. This encodes an amino acid at position 216 (arginine) that is different from the reference (glycine) and may have implications on structure and or function of the resulting 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>IL2RG (Xq13.1)</td>
<td>Negative</td>
<td>No known pathogenic variant detected in these genes</td>
</tr>
<tr>
<td>IL7R (5p13.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

INDICATIONS FOR TESTING
Individuals with a diagnosis of Severe Combined Immunodeficiency (SCID) with genetic counseling, are candidates for testing.

METHODOLOGY
Gene sequencing: All coding exons and associated intron junctions are analyzed by direct DNA sequence analysis using an automated fluorescent sequencing machine. When a mutation is detected, confirmation is carried out on an independent amplification of PCR using a second prep (B-prep) by sequencing in the opposite direction. If no mutation is found, sequence analysis is performed in both directions.
PERFORMANCE
Gene sequencing: From previous experience, we have been able to detect ADA, IL2RG, or IL7R mutations in about 99% of individuals with the diagnosis of Severe Combined Immunodeficiency (SCID) with specificity of mutation detection in probands detection is also estimated to be greater than 99%.

LIMITATIONS
The sequence analysis will not detect mutations located in regions of ADA, IL2RG, or IL7R that are not analyzed (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions). The sequencing method also will not detect gross genetic alterations including most duplications, inversions, or deletions (in females). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

CLINICAL DESCRIPTION
Group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. It is inherited as an X-linked or autosomal recessive defect. Mutations occurring in many different genes cause human Severe Combined Immunodeficiency (SCID).

-from MeSH
**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:
   
   "Severe combined immunodeficiency disease"[ExactTitle]

   In the “Term Hierarchy” section you can see more specific sub-types of SCIDS -two major forms are displayed. Click the names of the diseases to open the MedGen records to read about each sub-type.

   **WHAT IS/ARE THE MAJOR DIFFERENCES IN THE TWO SUB-TYPES OF SCIDS?**

   **WHICH ONE WAS SUSPECTED IN ASHANTI?**

   **WHY MIGHT YOU HAVE SELECTED THIS ONE AS MOST LIKELY, EVEN IF YOU DIDN’T HAVE THE LAB RESULTS?**

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**Understanding the Genetic Test Results**

2. **WHAT IS THE SPECIFIC GENE AND VARIATION IDENTIFIED IN ASHANTI?**
   
   (Read the results, sometimes it is really helpful!)

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

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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: **ADA Gly216Arg**
INFORMATION ABOUT THIS GENE FROM HUMAN-CURATED SOURCES:

3. On the MedGen record, **click the link for the gene** identified as having a variant in Ashanti.  
   **What does this gene normally do?**

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).
   **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?**
   **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.
   **In which tissues has this gene been found to be expressed?**

   **Do any of these tissues correlate with what may be malfunctioning in Ashanti?**
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.

WHERE IS ASHANTI’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)

7. On the RefSeqGene page, (on the right-hand side) you can click the “Protein” link, select Adenosine Deaminase Isoform 1 (the longest one) and click “Graphics” to see a graphical view of the annotated regions curated on the protein sequence. The information shown in in these “tracks” of this view can help you to learn more about this protein.

WHERE IN THE PROTEIN SEQUENCE IS ASHANTI’S GENETIC VARIANT LOCATED?
(On the picture above or on your screen – draw or visualize a vertical line at the position of each of the variants. You can learn more about the main functional regions of the protein click “Identify Conserved Domains”.)

WHAT MIGHT BE THE IMPACT OF THE GENETIC VARIATION ON THE PROTEIN’S FUNCTION?
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 ADA protein (PDB accession: 3IAR) as displayed in NCBI’s Cn3D Viewer.

The protein backbone is showed in a long light blue tube. The active site residues are shown in yellow (with side-chains displayed in yellow ball-and-stick rendering) surrounding a Zinc ion cofactor and an adenosine substrate analog shown in spacefill rendering.

The Gly216 position is shown in a pale yellow and exists in the tight turn of a loop which holding together two key residues that are critical for binding to the Zinc and catalyzing the reaction.

The inset is a zoomed-in view of the active site. What do you think the change in amino acid might do to the 3D structure and function of the protein?

### Gly216

Gly216 has a flexible backbone with a tiny side chain (a proton) that barely extends into a hydrophobic pocket within the protein. This enables the backbone’s tight bend enabling the neighboring residues to tightly surround the active site and participate in binding and catalysis.

Substitution of a glycine with a large, charged arginine side chain (Gly216Arg) would completely disrupt the turn and blow apart the hydrophobic pocket perturbing/disrupting the entire structure. This causes the enzyme to unfold and lose all activity.

In your Nucleotide Metabolism Session (Dr. Elliott - Week 8) you will learn: how the loss of the adenosine deaminase activity causes the build-up of toxic levels of deoxyadenosine, which interferes with DNA replication and promotes apoptosis - killing fast growing cells.

How does this help to explain the molecular pathology causing Ashanti’s Disease? (Which cells was this expressed in and are subsequently killed off? hint: see #5)
SUMMARY QUESTIONS – You will be asked to discuss these specific questions on Friday.

Introduce your patient to the class!

Who is she? What is her story?
(see the referral form)

What was the preliminary diagnosis and the rationale for it?
(see the referral form)

What did the genetic test find and how does this relate to the preliminary diagnosis?
(see the genetic test result form)

What is the implicated/affected gene and what is its normal function?
(NCBI’s Gene database should help!)

Where in the gene and gene product is the patient’s genetic variant located?
(Where in the gene? In what part of the mRNA? Where in the protein? In what functional part of the protein?)

What is the molecular impact of the genetic variant on the gene product?
(What do you think the variant ended up doing to the protein structurally?)

What do you think might be the functional impact of the variant on the gene product and in the patient?
(What impact do you think the variant had on the function of the protein? How might this relate to the patient’s symptoms?)

Now that you’re done.....SELF-ASSESSMENT TIME!

My initial ideas about this 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?)

What specific content areas do I need to review?