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.
Patient Information

Patient Name

JILL

DOB, Medical Record Number (MRN)


Requesting Provider

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

Specialty/Department:
Internal Medicine

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 Congenital Muscular Dystrophy (LMNA) and Lipodystrophy

Diagnosis: G71.2 – Congenital Muscular Dystrophy & E88.1 Lipodystrophy, not elsewhere classified

Clinical Notes: 19 year old female was diagnosed as a young child with Congenital Muscular Dystrophy (CMD). At the age of 12 her parents became concerned as she began to develop a “skeletal appearance”, but it was attributed to having “picky eating habits”. She has come to you with the results of a research project that she initiated as a Biology course project in her first year at college. She believes that she may have a secondary lipodystrophy disorder arising from the malfunction of a common CMD gene. She has asked for genetic screening of the LMNA gene to identify specific genetic variants that might be causing either one or both diagnoses. (And she could use this for her undergraduate thesis….)

A blood sample has been sent out for analysis with a CMD genetic testing panel. The genetic test result report will be faxed to the Molecular Science/M1 Training program for evaluation.

Please consult with the patient 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. 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 5:10 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>LMNA (1q22)</td>
<td>Arg453</td>
<td>This result confirms the diagnosis of Limb-Girdle Muscular Dystrophy type 1B. 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 Arg453Trp (LMNA: g.58841C&gt;T, c.1357C&gt;T or p.Arg453Trp) variation. The Arg453Trp mutation is caused by a C to T change at nucleotide position 58841 in the LMNA gene. This encodes an amino acid at position 453 (tryptophan) that is different from the reference (arginine) 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></td>
<td>Arg453Trp</td>
<td></td>
</tr>
<tr>
<td>LMNA (1q22)</td>
<td>Arg482</td>
<td>This result suggests a diagnosis of Familial Partial Lipodystrophy, type 2. 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 Arg482Trp (LMNA g.59412C&gt;T, c.1444C&gt;T or p.Arg482Trp) variation. The Arg482Trp mutation is caused by a C to T change at nucleotide position 159412 in the LMNA gene. This encodes an amino acid at position 482 (tryptophan) that is different from the reference (arginine) 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></td>
<td>Arg482Trp</td>
<td></td>
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</tbody>
</table>
INDICATIONS FOR TESTING
Individuals with a diagnosis of Emery-Dreifuss Muscular Dystrophy 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 EMD, FHL1, LMNA, SYNE1, SYNE2, or TMEM43 mutations in about 99% of individuals with the diagnosis of Emery-Dreifuss Muscular Dystrophy 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 EMD, FHL1, LMNA, SYNE1, SYNE2, or TMEM43 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. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

CLINICAL DESCRIPTION
Emery-Dreifuss muscular dystrophy (EDMD) is characterized by the clinical triad of joint contractures that begin in early childhood, slowly progressive muscle weakness and wasting initially in a humero-peroneal distribution that later extends to the scapular and pelvic girdle muscles, and cardiac involvement that may manifest as palpitations, presyncope and syncope, poor exercise tolerance, and congestive heart failure. Age of onset, severity, and progression of muscle and cardiac involvement demonstrate both inter- and intrafamilial variability. Clinical variability ranges from early onset with severe presentation in childhood to late onset with slow progression in adulthood. In general, joint contractures appear during the first two decades, followed by muscle weakness and wasting. Cardiac involvement usually occurs after the second decade.

-from GeneReviews

Familial partial lipodystrophy type 1 (FPLD1), or Kobberling-type lipodystrophy, is characterized by loss of adipose tissue confined to the extremities, with normal or increased distribution of fat on the face, neck, and trunk (Kobberling and Dunnigan, 1986). For a general description and a discussion of genetic heterogeneity of familial partial lipodystrophy (FPLD), see 151660.

-from OMIM
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: "muscular dystrophy" AND LMNA[gene]

   **WHAT ARE THE FOUR MUSCULAR DYSTROPHY SUBTYPES THAT ARE ASSOCIATED WITH GENETIC VARIATIONS IN THE LMNA GENE?**

   Genetic testing might provide some answers about which one you should focus on.

   **WHEN YOU HAVE AN ANSWER…..RETURN HERE TO FIND A HELPFUL RECORD (PERHAPS TWO) AND LEARN MORE!**

Understanding the Genetic Test Results

2. **WHAT IS THE SPECIFIC GENE AND VARIATION ASSOCIATED WITH MUSCULAR DYSTROPHY FOUND IN JILL?** (Read the results, sometimes it is really helpful!)

   **WHAT DOES THE GENETIC TEST RESULT SAY ABOUT A SPECIFIC MUSCULAR DYSTROPHY TYPE?**

   It appears that a “Secondary Finding” is listed. This is when a variant is “found” that wasn’t specifically requested or designated to be included for analysis. It is something that happens on occasion and increasingly so when large panels of genes or full gene/exome sequencing occurs. There is some controversy about reporting Secondary Findings. In this case, Jill was well informed and wanted to know anything that came out of the testing.

   **WHAT IS THE OTHER SPECIFIC GENE AND VARIATION FOUND?**

   **WHAT DIAGNOSIS DOES THIS SECONDARY FINDING SUGGEST?**

   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: LMNA Arg453Trp OR LMNA Arg482Trp
3. On the MedGen record, click the link for the gene identified as having variants in the Jill. **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 and, since the protein is maintained within the cell, where it functions.
   **In which tissues has this gene been found to be expressed?**

While Jill is obviously intellectually “normal” (well, really pretty darn brilliant), many of her organ systems are affected, most notably muscles and fat/adipose, but also and others, such as her lungs and skin.

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

**How many transcript variants and encoded proteins** (often called isoforms) **are known to be produced by this gene?**

**Where are Jill’s 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.
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 positions of the two variants in the gene, what is the most likely mechanism for impacting the final gene product for each?** *(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 Lamin Isoform A (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 ARE JILL’S 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.)

**IN WHICH CDD DOMAIN ARE THESE GENETIC VARIATIONS LOCATED?**
(You can learn more about the main functional regions of the protein click “Identify Conserved Domains” and then you can mouse-over the domains or click on them to learn what PFam Domain Curators have annotated.)

One of the things that is noted about the LTD region is that it has an Ig-fold structure (“beta-sandwich” held together a disulfide bond) that has two main faces.

**WHAT MIGHT BE THE IMPACT OF THE GENETIC VARIATIONS 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** of the LTD Domain of the Human LMNA protein (PDB accession: 3GEF) as displayed in NCBI’s Cn3D Viewer.

The protein backbone and beta-sheet bars are showed in a long **light blue** tube, with Arg453 and Arg482 shown as spacefill rendering extending outward from the surface of either side of the Ig-fold structure (“beta-sandwich”).

These surfaces serve as a “staging center” or docking-port for Transcription factors and other proteins to bind awaiting activation. Upon which time, they are released and can make their way to the chromatin to activate gene expression.

**WHAT DO YOU THINK THE CHANGE IN AMINO ACIDS MIGHT DO TO THE SURFACE OF EACH SIDE OF THE DOMAIN’S 3D STRUCTURE?**

The picture to the left is an overlay of two LMNA LTD domain structures:
- one containing a normal Arg482 (shown in blue indicating it is charged and hydrophilic and sticking out from the surface) and
- the other containing the variant Trp482 (shown in brown - pseudo-aromatic, hydrophobic side chain…and folded closer to the surface)

A substitution of either arginine with a tryptophan would cause both physical (shape) and chemical (charge, polarity) differences in the surface. This would dramatically impact the ability of an interacting protein (such as a Transcription factor) to dock to the “staging center”.

Jill was not alone. She deduced from what she learned about her two disorders that one domain face was likely involved in her muscular dystrophy (muscle-wasting disease), while the other was likely involved in her lipodystrophy (fat-wasting disease).

Unlike Jill (who had neither much muscle or fat), her father seemed to have “Popeye arms”. They were very muscular with little fat overlay, so that the muscle striations were visible. She read that the lipodystrophy could result in heart attacks due to high levels of circulating lipids (there was limited adipose tissue to be stored in). She begged her father to go get it checked out…..and it saved his life.

**THERE’S MORE TO JILL’S STORY AND WE’LL TALK ABOUT IT ON FRIDAY.**
**SUMMARY QUESTIONS** – You will be asked to discuss these specific questions on Friday.

<table>
<thead>
<tr>
<th>Introduce your patient to the class!</th>
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<tbody>
<tr>
<td>Who is she? What is her story?</td>
</tr>
<tr>
<td>(see the referral form)</td>
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</table>

| 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?) |

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

<table>
<thead>
<tr>
<th>My initial ideas about this case:</th>
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<tbody>
<tr>
<td>(Why did I think this? How confident was I?)</td>
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<table>
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<tr>
<th>What did I miss?</th>
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</thead>
<tbody>
<tr>
<td>(Why did I miss it? How could I have thought about it differently?)</td>
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<tr>
<th>What specific content areas do I need to review?</th>
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