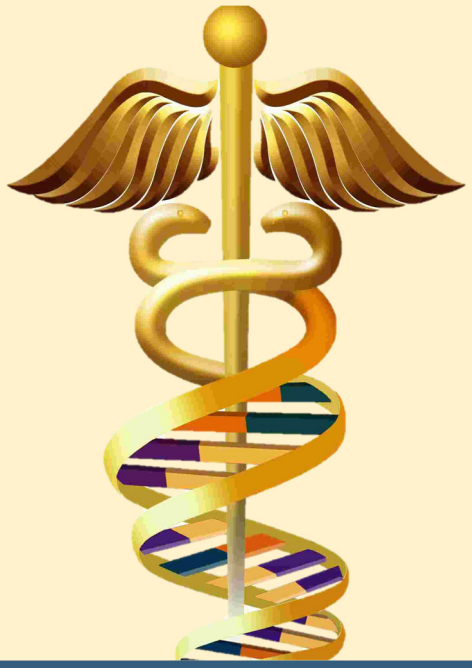
Foundations of Medicine

Sessions 164 & 167

Group Case



Case Studies

Making connections between Genetics, Molecular Biology, & Biochemistry

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 discussed the state of clinical practice with regard to the application of Precision Medicine principles (examining a patient's specific molecular pathology).
- Together we explored a real-world case study and followed a workflow to discover the patients' molecular pathology for an undiagnosed/misdiagnosed problem.

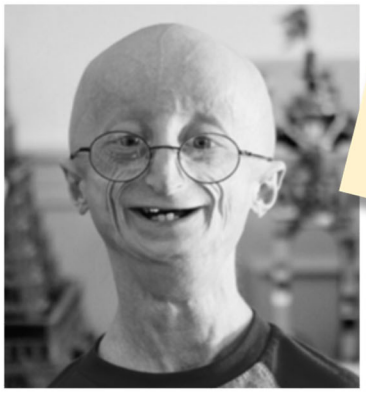
Before Friday's Session:

- **Your group has your own case study to solve!**

In Friday's Session:

- We will discuss each group's case and discover the underlying cause of pathology in these real patients.
- We'll compare what is happening at the molecular level in other patients that have seemingly related cases.

Sam



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

MyClinicalService

Physician Referral Form

Patient Information	
Patient Name **SAM**	Patient Barcode Sticker 
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 Hutchinson-Guilford Progeria**

Diagnosis: **E34.8 – Other specified endocrine disorders**

Clinical Notes: 22 month old boy apparently healthy for the first 6 months of his life began deteriorating in growth (height and weight) and showed signs of alopecia. However, tests for alopecia and other evaluations were negative. Over the next year the parents (both physicians) pushed for further evaluations and at 22 months, he was diagnosed with Hutchinson-Guildford Progeria. The mother has now gone into medical research and, working with a human genetics expert, is attempting to identify the molecular mechanism behind his disorder which has been narrowed down to a defect in the LMNA protein - for development of a specific and effective treatment if not cure.

A blood sample has been sent out for analysis with an Hutchinson-Guildford Progeria (LMNA) 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 6:17 PM EDT

ClinGenLab
Clinical Testing Laboratory, Inc.

Clinical Testing Lab of Washington
2150 Pennsylvania Avenue NW
Washington, DC 20037
Phone: 202-555-1212

Specimen Number	Specimen Type	Control Number	Account Number	Account Phone Number	Route
	Peripheral Blood				

Patient Last Name	Patient Barcode

Patient First Name	Patient Middle Name
Sam	

Patient SS#	Patient Phone	Total Volume

Age (Y/M/D)	Date of Birth	Sex	Fasting
22 m.o.		Male	

Patient Address	Indication: Progeria Family History: No family history Ethnicity: Western European Caucasian

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
			Jane Ferreiro, MD		

Tests Ordered
Hutchinson-Gilford Progeria Syndrome (HGPS) via the LMNA Gene

General Comments
Please send a copy of the final report to the Molecular Science/M1 Training office via Fax at (202) 555-1212

Clinical test results for Severe Combined Immunodeficiency (SCID)

GENE	TEST RESULTS	EXPLANATION
LMNA (1q22)	Gly608 Gly608=	**This result confirms the diagnosis of Hutchinson-Gilford Progeria Syndrome (HGPS)**. 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 Gly608= (LMNA: g.61041C>T, c.1824C>T or p.Gly608=) variation. The Gly608= mutation is caused by a C to T change at nucleotide position 1824 in the LMNA gene. While this does not result in an altered encoded amino acid (=), it has been reported that the nucleotide variant impacts post-transcriptional processing of the mRNA transcript. The presence of the variant induces the use of a novel/cryptic splice donor site within exon 11 at position 1818. This is ligated directly to the reference splice acceptor site of exon 12, resulting in the deletion of encoded amino acid residues 607 to 656. Furthermore, loss of this protein region has been shown to prevent full post-translational processing (proteolytic cleavage) of the 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.

INDICATIONS FOR TESTING
Individuals with a diagnosis of Hutchinson-Gilford progeria syndrome 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 LMNA mutations in about 99% of individuals with the diagnosis of Hutchinson-Gilford progeria syndrome 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 LMNA 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
Hutchinson-Gilford progeria syndrome encompasses a spectrum of clinical features that typically develop in childhood and resemble some features of accelerated aging. Although signs and symptoms vary in age of onset and severity, they are remarkably consistent overall. Children with Hutchinson-Gilford progeria syndrome (HGPS) usually appear normal at birth. Profound failure to thrive occurs during the first year. Characteristic facies, with receding mandible, narrow nasal bridge and pointed nasal tip develop. During the first to third year the following usually become apparent: partial alopecia progressing to total alopecia, loss of subcutaneous fat, progressive joint contractures, bone changes, nail dystrophy, and abnormal tightness and/or small soft outpouchings of the skin over the abdomen and upper thighs, and delayed primary tooth eruption. Later findings include low-frequency conductive hearing loss, dental crowding, and partial lack of secondary tooth eruption. Additional findings present in some but not all affected individuals include photophobia, excessive ocular tearing, exposure keratitis, and Raynaud phenomenon. Motor and mental development is normal. Death occurs as a result of complications of severe atherosclerosis, either cardiac disease (myocardial infarction) or cerebrovascular disease (stroke), generally between ages six and 20 years. Average life span is approximately 14.6 years.

-from GeneReviews

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:** **`progeria AND LMNA`**

Understanding the Genetic Test Results

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

   WHAT DOES THE GENETIC TEST RESULT MEAN FOR SAM'S DIAGNOSIS?

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 Gly608=`

Molecular Biology Research

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 Sam.
   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?

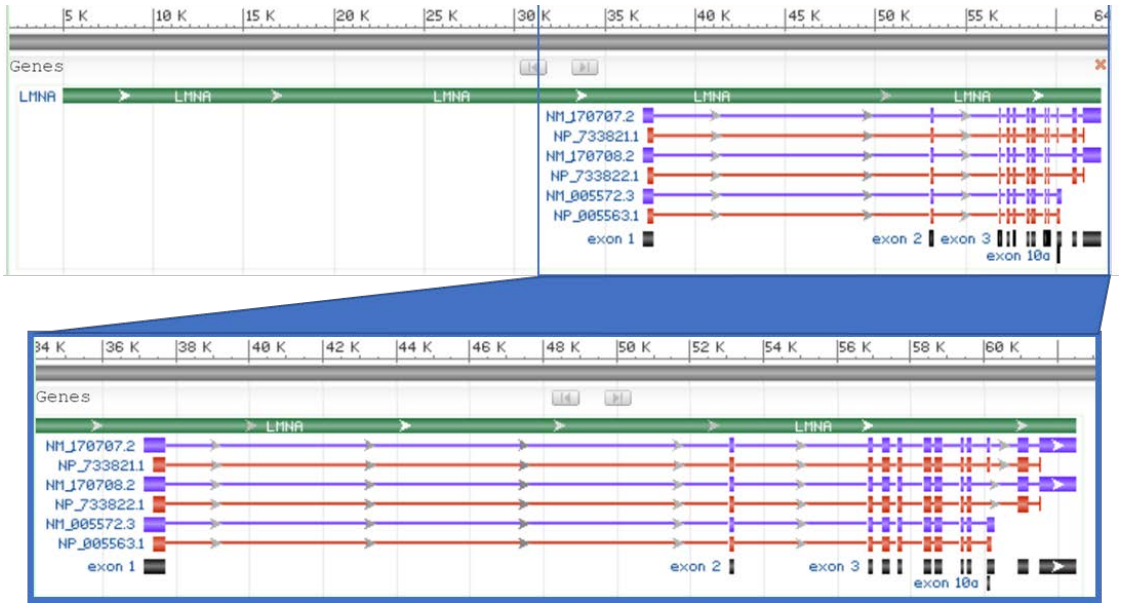
IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?

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?

DO ANY OF THESE TISSUES CORRELATE WITH WHAT MAY BE MALFUNCTIONING IN SAM?

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 SAM'S GENETIC VARIANT LOCATED IN THIS GENE AND IN THE MRNA (WHICH EXON)?
(On the picture above or on your screen – draw or visualize a vertical line at the position of each if the variants.

Your first thought would be that, since this gene variant is located in the coding region of an exon, this genetic variant should impact the protein at the encoded amino acid position...*but the amino acid doesn't change!*

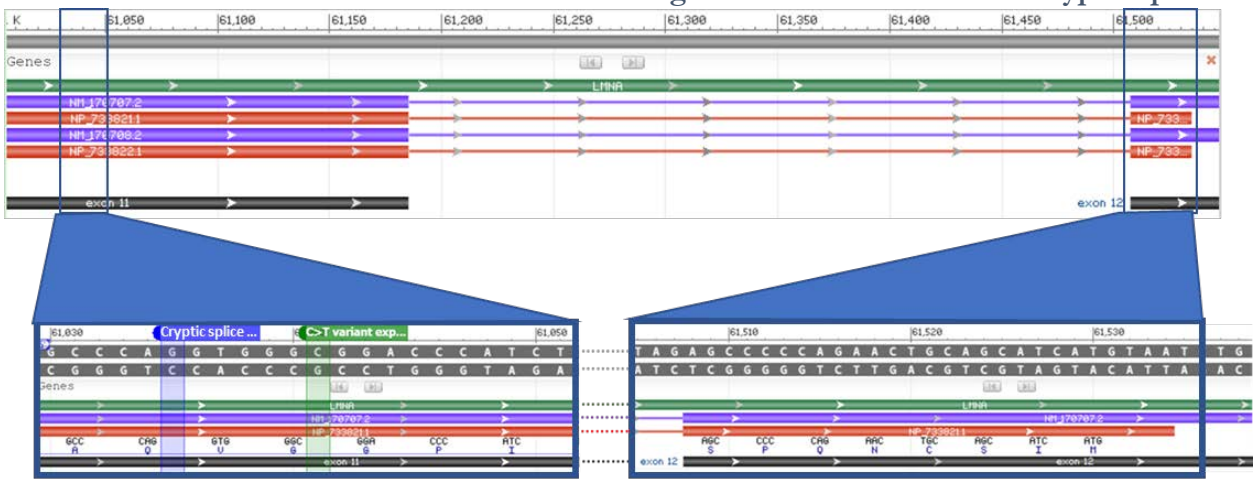
In reality, it took quite a bit of work in a research lab to figure out and prove what was going on with Sam's genetic variant. In fact, his mother (a physician) joined a renowned research lab to work on this. It became her life's work and she has now become a world's expert on this disease and, since she figured out what is happening on the molecular level – she is now actively working on treatments and a cure.

Zoom in on the region around the variant's exon and the one it normally connects up with.
(You can type in the variant's gene position, from the genetic test result, into the "Find" box to automatically zoom in!)

HOW DOES THE "NORMAL" SPLICING BETWEEN THESE EXONS OCCUR?

In a very unusual situation, "normal splicing" between these two exons does not always occur in LMNA transcripts. Even in those with "wildtype" or normal sequences, the 3D structure of the RNA transcript is such that a "Cryptic splice site" (position 61035) within exon 11 can be used instead of the normal one.

Zoom in a bit further to see the bases around Sam's genetic variant to see the "Cryptic splice site".



In Sam's case, and in virtually all other kids with his disorder, a genetic variant near this "Cryptic splice site" causes a shift in that 3D RNA structure which increases the use of this "Cryptic splice site" to serve as a splice donor site to connect with exon 12.

WHAT DO YOU THINK USE OF THE "CRYPTIC SPLICE SITE" AS THE SPLICE DONOR TO CONNECT TO EXON 12 DOES TO THE FULLY PROCESSED MRNA SEQUENCE?

WHICH AMINO ACID OF EXON 11 IS CONNECTED TO WHICH AMINO ACID OF EXON 12?

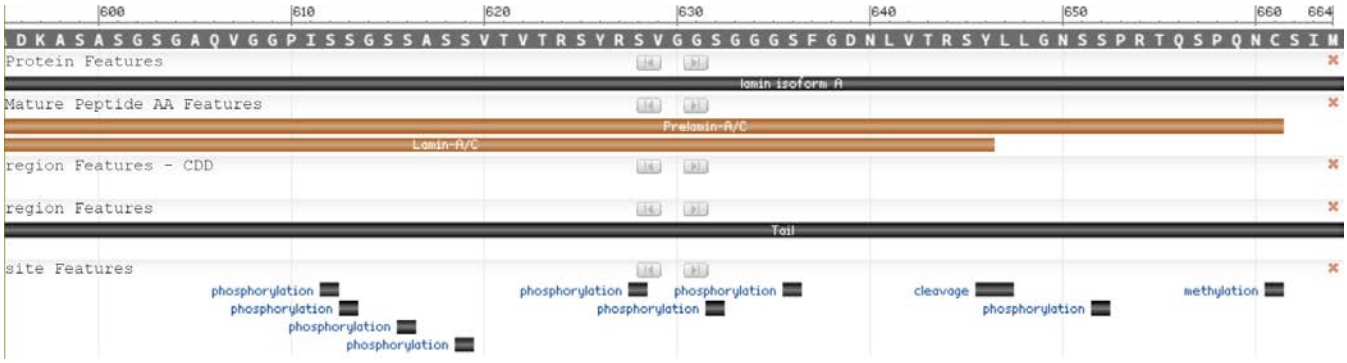
(**HINT**: The protein coding sequence frame of the mRNA is not shifted, so you don't need to worry about that. Also, Sam's variant indicated that his affected codon was at position Gly608- the Cryptic splice site is upstream of that.)

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 FULL-LENGTH PROTEIN SEQUENCE, ARE THOSE TWO AMINO ACID POSITIONS?

Zoom in a bit further to see the amino acids at the tail end of the normal LMNA protein.

In the picture below or virtually on your screen, draw lines for the location of each of these two amino acid positions you identified above.



IF THE POSITION OF THESE TWO AMINO ACIDS ARE SPLICED TOGETHER, WHAT IS MISSING FROM THE FINAL ENCODED PROTEIN?

8. In order to perform its normal functions, the LMNA protein (Lamin A) is moved to the nuclear inner membrane and then migrates to form a 2D matrix of proteins supporting nuclear stability, chromatin structure and gene expression.

   This is a multi-step process:

   1) Lamin A proteins are translated in the cytosol and moved into the nucleus.
      Look at the picture of the full-length protein sequence above *(previous page).*
      CAN YOU FIND A "MOTIF" THAT WOULD HELP TARGET THE LMNA PROTEIN TO THE NUCLEUS?
      (**HINT**: Do you remember Dr. Ellott's Protein Targeting session?)

   2) Then, a Farnesyltransferase attaches a farnesine group to the "C" near the very tail end. Then, the same "C" is targeted by Carboxymethyltransferase to replace the very end of the protein with a terminal methyl group. This assists in localization and anchors the Lamin A protein to the nuclear membrane.
      Look at the picture of the zoomed-in protein tail sequence above.
      CAN YOU FIND THE AMINO ACID TARGET FOR ATTACHING/CREATING THE MEMBRANE ANCHOR?

   3) Finally, the Lamin A protein is cleaved (cut by a protease) from the membrane anchor and released to migrate and help to form the inner membrane matrix – where it performs all sorts of functions.
      Look at the picture of the zoomed-in protein tail sequence above.
      CAN YOU FIND THE AMINO ACID TARGET FOR PROTEOLYTIC CLEAVAGE?

LET'S TIE THIS ALL TOGETHER WITH WHAT IS HAPPENING IN SAM.
His Lamin A protein is made, moved into the nucleus and attached to the nuclear inner membrane. But, due to the activation of the Cryptic splice site by his genetic variant, the protein is missing the portion enabling it to be released from the membrane surface….so it begins to form the matrix and bind chromatin and other proteins at the nuclear membrane surface - causing all sorts of problems.

WHAT DO YOU THINK THIS WOULD TRIGGER THE CELL TO DO IN RESPONSE AND WHICH TISSUES *(see #5)* **MIGHT THIS AFFECT?**

HOW MIGHT THIS RELATE TO SAM'S SYMPTOMS/CLINICAL FEATURES?

SUMMARY QUESTIONS – ***You will be asked to discuss these specific questions.***

Introduce your patient to the class!

Who is he? What is his story?
(see the referral form)

What was the preliminary diagnosis and the rationale for it?
(see the referral form & NCBI's MedGen database)

What did the genetic test find and how does this relate to the preliminary diagnosis?
(see the genetic test result form & NCBI's ClinVar database)

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?