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.

James

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 **JAMES**	Patient Barcode Sticker 
DOB, Medical Record Number (MRN)	

Requesting Provider	
Assigned Provider/Practice Name: Jane Ferreiro, MD / MyClinicalService	Specialty/Department: Family Practice
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 Hemophilia**

Diagnosis: **D66 – Hemophilia A (Factor VIII Deficiency)**

Clinical Notes: 8 month old boy was referred after a visit to the emergency room due to a relentless nosebleed caused by a fall, "bumping into a coffee table". His mother was also concerned about some visible bruising on his knees and palms since he began crawling at 6 months. When questioned, the mother was concerned about a possible family history of bleeding issues. She required a blood transfusion after natural childbirth, which her doctor suggested was unusual, and her only brother died at the age of 6 years old from a "brain bleed" after he fell out of a tree in the family back yard.

A lab test suggested a deficiency in Factor VIII and a blood sample has been sent out for analysis with a Hemophilia genetic testing panel. The genetic test result report will be faxed to the Molecular Science/M1 Training program for evaluation.

Please consult with the mother 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 12:57 AM 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
James	

Patient SS#	Patient Phone	Total Volume

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

Patient Address	Indication: Hemophilia Family History: Family history of uncontrolled bleeding

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

Tests Ordered
Hemophilia Mutation Evaluation

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 DNA Hemophilia A Mutation Evaluation

GENE	TEST RESULTS	EXPLANATION
F8 (Xq28)	Arg15Ter	**This result confirms the diagnosis of Hemophilia A**. This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., APTT, Factor VIII Activity, etc.). A PCR/sequencing study has detected one copy of the Arg15Ter (F8:g.5214C>T, c.43C>T or p.Arg15Ter) variation. The Arg15Ter variation is a C to T change at nucleotide position 5214 of the F8 gene and 43 of the F8 mRNA transcript. This forms a premature stop (termination) codon at amino acid position 15 resulting in an abnormally short or truncated protein. As males have only one copy of the X chromosome, a variation in an X-linked gene renders the patient with only a mutated form of the gene. Thus, they are susceptible to the most severe form of the disease.
F9 (Xq27.1)	Negative	

INDICATIONS FOR TESTING
Individuals with a diagnosis of hemophilia A, appropriate at-risk female relatives of probands with identified mutations, and hemophilia A carriers with genetic counseling, are candidates for testing.

METHODOLOGY
Factor VIII sequencing: All coding exons and associated intron junctions of the Factor VIII gene 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
Factor VIII sequencing: From previous experience, we have been able to detect factor VIII gene mutations in about 99% of individuals with the diagnosis of hemophilia A with specificity of mutation detection in probands and carrier detection is also estimated to be greater than 99%.

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**: `Hemophilia[ExactTitle]`

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

   WHAT IS/ARE THE MAJOR DIFFERENCES IN THE TWO SUB-TYPES OF HEREDITARY HEMOPHILIA?

   WHICH ONE WAS SUSPECTED IN JAMES?

Understanding the Genetic Test Results

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

   THEY ONLY IDENTIFIED ONE COPY OF A VARIANT IN THE GENETIC TEST RESULTS. WHY DO YOU THINK THAT IS SO?

   WHAT DOES THE GENETIC TEST RESULT SAY THIS MEANS FOR JAMES' 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:

`F8 Arg15Ter`

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 James.
   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?

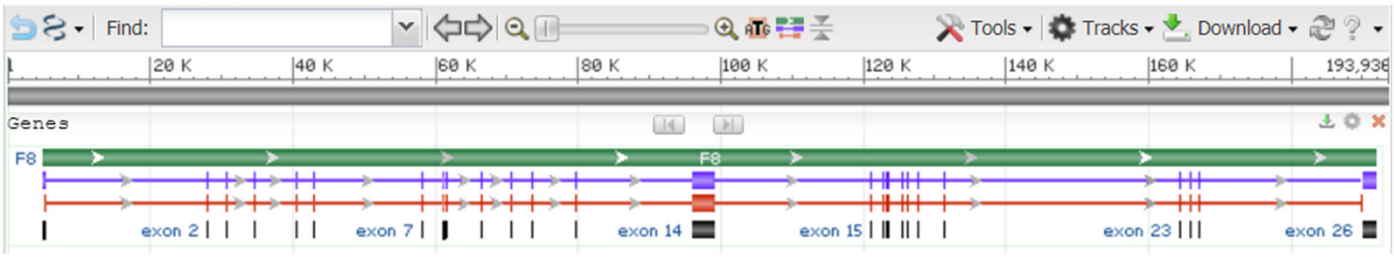
   IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?

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?

   BASED ON WHAT YOU READ ABOVE, ABOUT THE FUNCTION AND PURPOSE OF THIS PROTEIN, WHAT WOULD YOU PREDICT TO FIND IN THE PROTEIN SEQUENCE? (**HINT:** if a protein is made in a cell type/tissue, but functions elsewhere….how does it get there? Ask Dr. Elliott if you *really* can't remember.)

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.



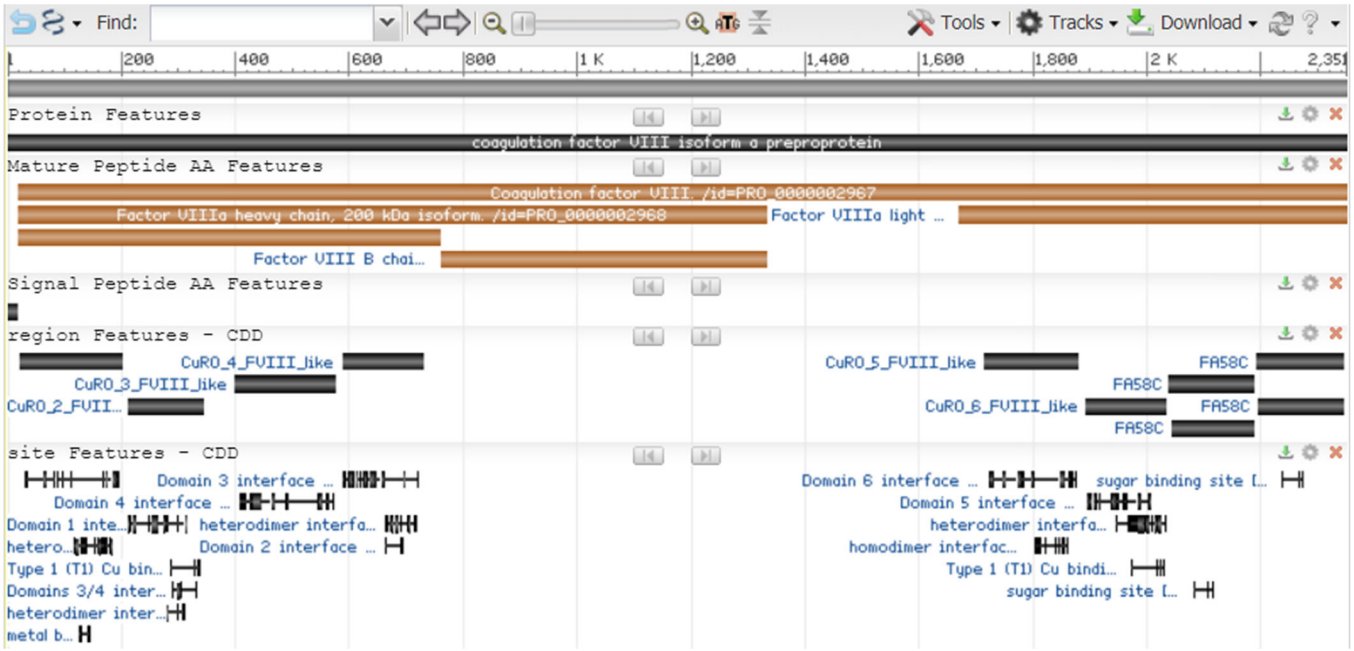
WHERE IS JAMES' GENETIC VARIANT LOCATED IN THIS GENE AND IN THE MRNA?
(On the picture above or on your screen – draw or visualize a vertical line at the variant's position.
You can type in the variant's gene position, from the genetic test result, into the "Find" box to automatically zoom in!)

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** or go back to the Gene record and **click the "RefSeq Proteins" link**.

   There are two transcripts created from this gene: a major one encoding the isoform A preprotein and a minor one that contains a unique 5' exon located within intron 22 of major transcript - which is spliced to encode a short peptide from exons 23-26. To see a graphical view for isoform A along with its annotated regions curated on the protein sequence, click "**coagulation factor VIII isoform a preproprotein [Homo sapiens]" then click "Graphics"** (under the title).

   The information shown in in these "tracks" of this view can help you to learn more about this protein.



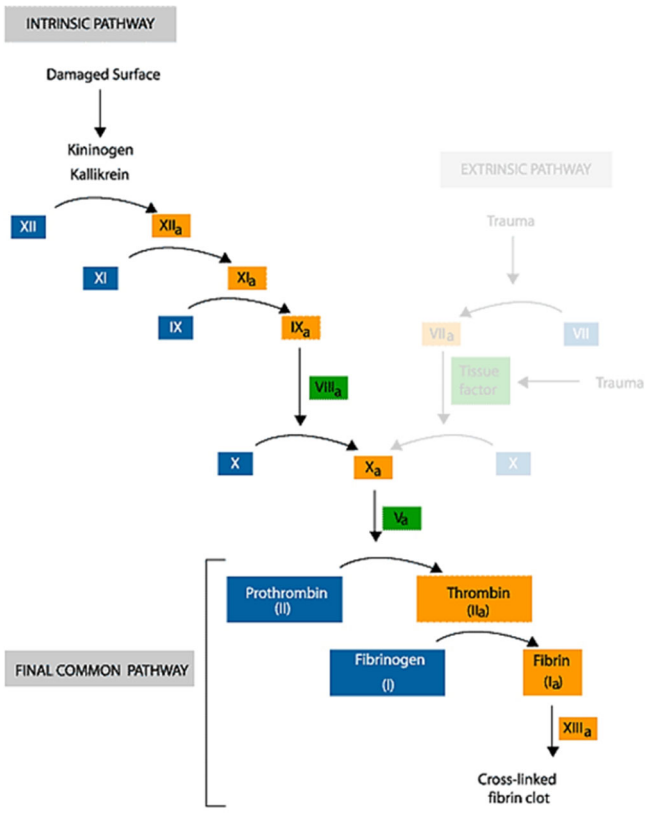
WHERE IN THE PROTEIN SEQUENCE IS JAMES' GENETIC VARIANT LOCATED?
(On the picture or on your screen – draw or visualize a vertical line at the position of each if the variants. You can learn more about the main functional regions of the protein click "Identify Conserved Domains"))

WHAT MIGHT BE THE IMPACT OF THE GENETIC VARIATION ON THE PROTEIN'S FUNCTION?

8. You found out earlier that this protein is not only involved, but critical for inducing the formation of a clot to stop bleeding.

   Here is a graphic of the Clotting Cascade pathway →

   HOW DO YOU THINK JAMES' GENETIC VARIANT ULTIMATELY IMPACTS HIS ABILITY TO STOP BLEEDING?



(Can you convert these Roman Numerals into Arabic Numerals to figure out which step is affected?)

WHY DO YOU THINK JAMES WAS SO SEVERELY AFFECTED BY THIS PARTICULAR GENETIC VARIATION?
(**HINT**: Think about both the impact of the particular variant on the protein and also what this means for James – as a boy. If you need to, go back and look at your notes for #7 & #2, respectively.)

SUMMARY QUESTIONS – ***You should be prepared 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?