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:

- **There is a practice case study to solve!**

In Friday's Session:

- You will work in groups to practice solving additional case studies as independent exercises – and we will discuss the cases 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.

Alexei

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

Requesting Provider	
Assigned Provider/Practice Name: Jane Ferreiro, MD / MyClinicalService	Specialty/Department: Pediatrics
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: **D68.311 – Hemophilia B (Factor IX Deficiency)**

Clinical Notes: 8 year old boy developed a severe hematoma on his left thigh after bumping into a boat's oarlock. His father indicated a long history of recurrent episodes of illness (bruises, bleeding episodes, and long painful recoveries) since shortly after birth.

The father's concern about his current condition is driven by the presence of a Monk with a questionable reputation who claims he alone and his mysticism can cure the boy. The father would like to take a more scientific approach and examine the possibility of an inherited bleeding disorder that appears to exist in many cousins of the maternal family.

A lab test suggested a deficiency in Factor IX 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 father 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 11:43 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
Alexei	

Patient SS#	Patient Phone	Total Volume

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

Patient Address	Indication: Hemophilia, possibly type B Family History: Family history of uncontrolled bleeding in males Ethnicity: Western European Caucasian

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 Mutation Evaluation

GENE	TEST RESULTS	EXPLANATION
F9 (Xq27.1)	c.278-3A>G	**This result supports the diagnosis of Hemophilia B**. This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., APTT, Factor IX Activity, Factor IX Inhibitor, etc.). A PCR/sequencing study has confirmed one copy of a F9 gene with putative splice-site variation (F9: g.15338A>G, c.278-3A>G). This variation is an A to G change at nucleotide position 15338 of the F9 gene. It has been reported that this nucleotide variant impacts post-transcriptional processing of the mRNA transcript. The presence of this variant induces the use of this position as a novel splice acceptor for exon 2, causing a -2bp frameshift. The new in-frame coding sequence of exon 2 produces an abnormal 11 amino acid peptide (ending in a termination codon) - resulting in an abnormally short, 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.
F8 (Xq28)	Negative	

INDICATIONS FOR TESTING

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

METHODOLOGY

Factor IX sequencing: All coding exons (1-8) and associated intron junctions of the Factor IX 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 IX sequencing: From previous experience, we have been able to detect factor IX gene mutations in about 99% of individuals with the diagnosis of hemophilia B with specificity of mutation detection in probands and carrier detection is also estimated to be greater than 99%.

LIMITATIONS

The sequence analysis will not detect mutations located in regions of the Factor IX gene 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

Hemophilia B is characterized by deficiency in factor IX clotting activity that results in prolonged oozing after injuries, tooth extractions, or surgery, and delayed or recurrent bleeding prior to complete wound healing. This is an X-linked recessive bleeding disorder with an incidence of about 1 per 30,000 live male births. Hemophilia B affects males, however, all male offspring will be normal, and although all female offspring will be obligatory carriers, they rarely have symptomatic bleeding. In contrast, female offspring of carriers of hemophilia B have a 50% chance of being carriers themselves, and each male offspring has a 50% chance of being affected.

The age of diagnosis and frequency of bleeding episodes are related to the level of factor IX clotting activity. In severe hemophilia B, spontaneous joint or deep-muscle bleeding is the most frequent symptom. Individuals with severe hemophilia B are usually diagnosed during the first two years of life; without prophylactic treatment, they may average up to two to five spontaneous bleeding episodes each month. Individuals with moderate hemophilia B seldom have spontaneous bleeding; however, they do have prolonged or delayed oozing after relatively minor trauma and are usually diagnosed before age five to six years; the frequency of bleeding episodes varies from once a month to once a year. Individuals with mild hemophilia B do not have spontaneous bleeding episodes; however, without pre- and post-operative treatment, abnormal bleeding occurs with surgery or tooth extractions; the frequency of bleeding may vary from once a year to once every ten years. Individuals with mild hemophilia B are often not diagnosed until later in life. In any individual with hemophilia B, bleeding episodes may be more frequent in childhood and adolescence than in adulthood. Approximately 10% of carrier females are at risk for bleeding (even if the affected family member has mild hemophilia B) and are thus symptomatic carriers, although symptoms are usually mild. After major trauma or invasive procedures, prolonged or excessive bleeding usually occurs, regardless of severity.

REFERENCES

1. Yoshitake S, Schach BG, Foster DC, et al: Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). Biochemistry 1985 July 2;24(14):3736-3750
2. Giannelli F, Green PM, Sommer SS, et al: Haemophilia B: database of point mutations and short additions and deletions-eighth edition. Nucleic Acids Res 1998 Jan 1;26(1):265-268
3. Ketterling RP, Bottema CD, Phillips JA 3rd, et al: Evidence that descendants of three founders constitute about 25% of hemophilia B in the United States. Genomics 1991 Aug;10(4):1093-1096

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 ALEXEI?

Understanding the Genetic Test Results

2. **WHAT ARE THE SPECIFIC GENE AND VARIATION IDENTIFIED IN ALEXEI?**
   (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 MEAN FOR ALEXEI'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:

`F9 c.278-3A>G`

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 Alexei.
   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?

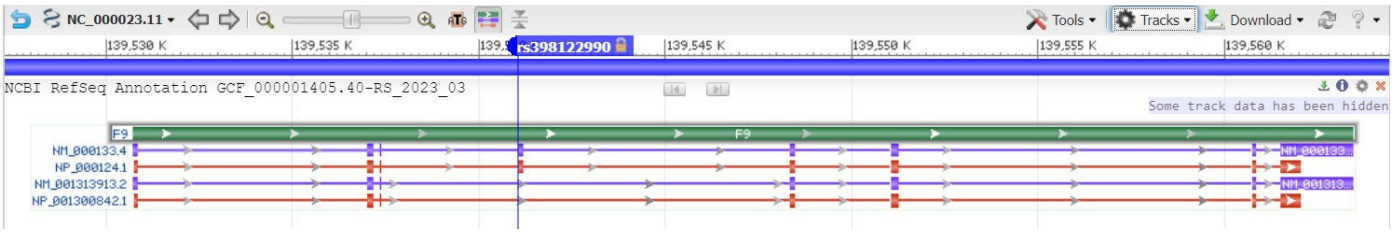
   IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?

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.)

MAPPING THE VARIANT THRUOGH THE CENTRAL DOGMA:

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.



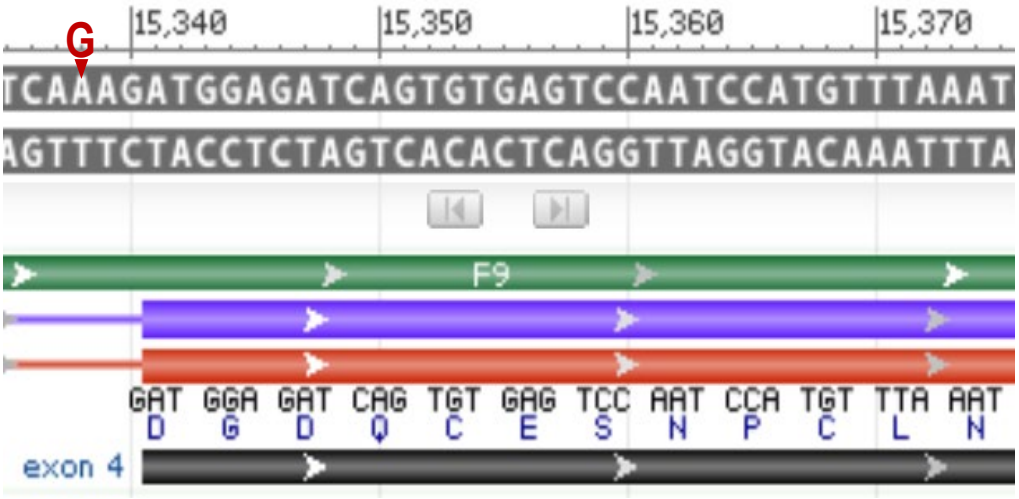
WHERE IS ALEXEI'S GENETIC VARIANT LOCATED IN THIS GENE AND IN THE MRNA?
(On the picture above or on your screen – draw or visualize a vertical line at the variant's position.)

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)*

Type the variant's gene position into the "Find" box to automatically zoom in!
Below is a zoomed-in picture showing the genome sequence (both strands) and the red "G" indicates the base change of the exact genetic variation next to the "-GA-" at position 15,340-15,341 which is the normal splice site acceptor indicated by the exon 4 **purple bar** with a "GAT" codon. The normal encoded amino acid sequence is shown below the **red bar**.

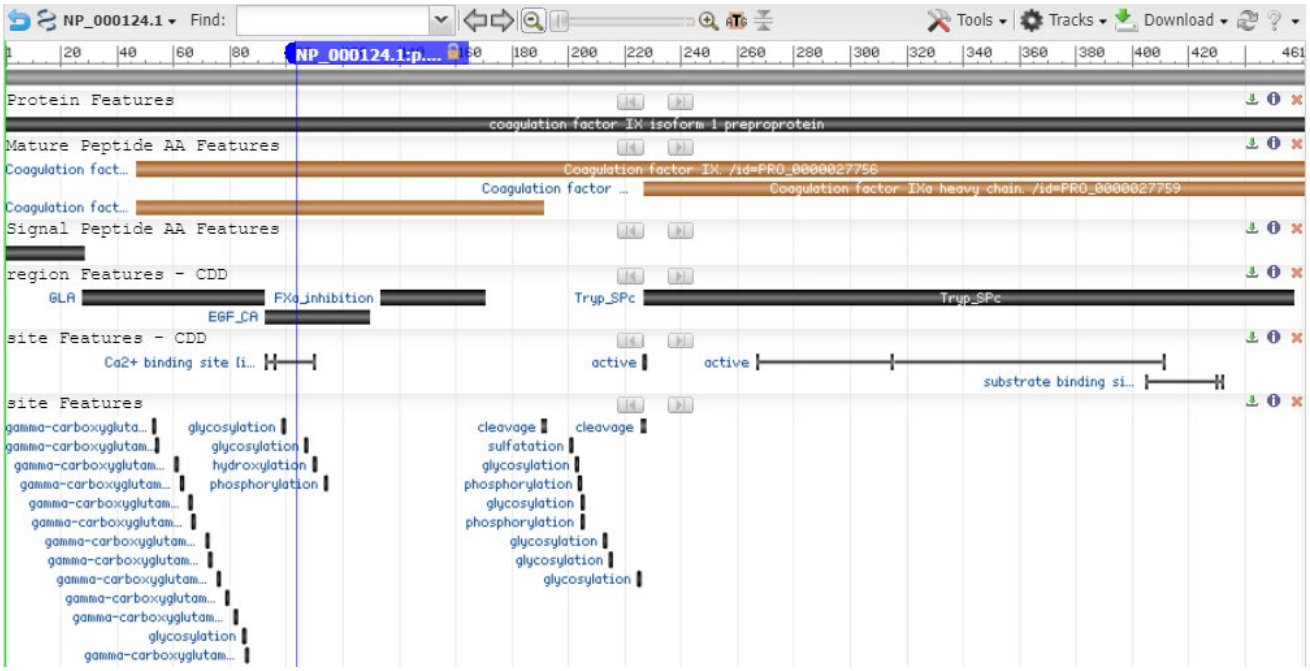
Alexei's genetic variation actually creates a new "-**G**A-" two letters down from the usual one….
which causes a -2 base frameshift in splicing and the start of the encoded amino acid sequence.

I *could* ask you to figure out what the new amino acid sequence would be…..starting with the new **G**AG codon, but instead…**Scan each 3 letter codon and find where there a "TAA" stop codon shows up up in the new frameshifted sequence.**



WHAT DO YOU THINK IS HAPPENING HERE TO THE FINAL FULL-LENGTH PROTEIN ENCODED BY THE GENE CONTAINING THE VARIANT?

7. On the Gene page, (on the right-hand side) **click the "RefSeq Proteins" link and then click "Graphics"** for the isoform1 version of this protein to see the annotated regions on the normal full-length protein. The information shown in in these "tracks" of this view can help you to learn more about this protein.



WHERE IS THE NEW STOP CODON IN THIS SCHEMATIC??
(On the picture above or on your screen – draw or visualize a vertical line at the position.)

WHAT IS THE IMPACT OF THE GENETIC VARIATION ON THE PROTEIN'S ABILITY TO FUNCTION?
(You can learn more about the main functional regions of the protein click "Identify Conserved Domains".)

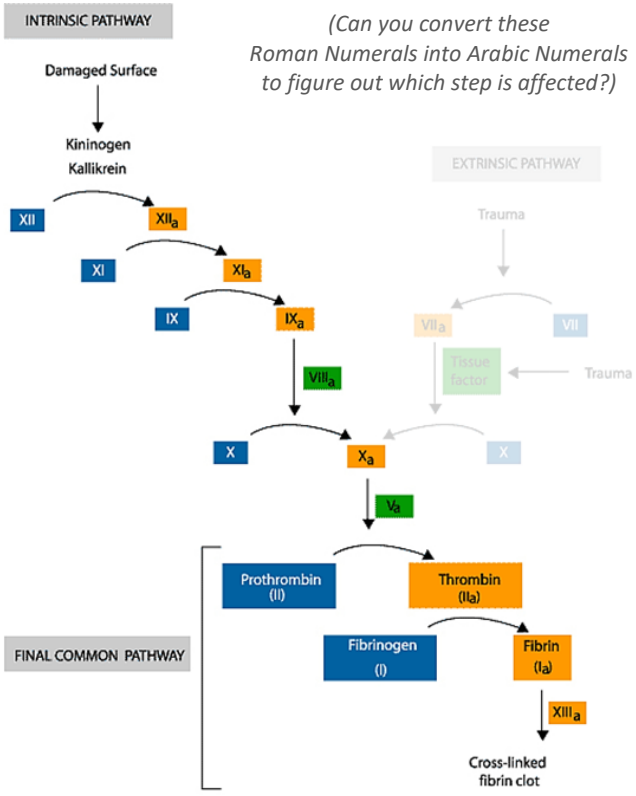
8. You found out earlier that this protein is not only involved, but critical for inducing the formation of a clot to stop bleeding.

   Here is a graphic of the Clotting Cascade pathway →

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

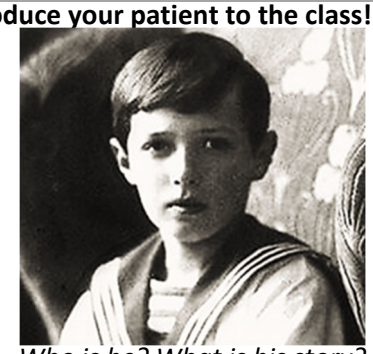
   WHY DO YOU THINK ALEXEI WAS SO SEVERELY AFFECTED BY THIS PARTICULAR GENETIC VARIATION?
   (Think about both the impact of the particular variant on the final protein and also what this means for James – as a boy. If you need to, go back and look at your notes for #6/7 & #2, respectively.)

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



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?