Welcome to FDN Session 164

"Discovering Molecular Mechanisms of Genetic Disorders with NCBI Resources"

Today's materials: https://bit.ly/2024foundations-molecularpathology

National Library of Medicine
National Center for Biotechnology Information

Workshop Materials
https://bit.ly/2024foundations-molecularpathology

A website with ALL of the information for today

and for you to refer to throughout med school.

National Library of Medicine
National Center for Biotechnology Information

NIH National Library of Medicine

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PRODUCTS AND SERVICES ▾   RESOURCES FOR YOU ▾   EXPLORE NLM ▾   GRANTS AND RESEARCH ▾

Home

Session Information
- Introduction
- Clinical Genetics/Genomics Toolkit: Research tips for patient work-ups
- Group Cases: Marco, Alexei, James & Bo
- Lightning Round: Speedy variant/phenotype research!
- Conclusion
- A Guided Case for Self-assessment: Leslie
- Independent Practice Cases for Self-assessment: Jeff, Jonathan, David

Share 𝕏 f in P

GWU Medical School Foundations of Medicine Block Session 164

Molecular Pathology Case Studies

Connecting the dots between genetics, molecular biology and biochemistry in real patients.

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 and has recently developed clinically-focused resources enabling scientists and clinicians to integrate known molecular biological information with clinically-relevant genetic variations.
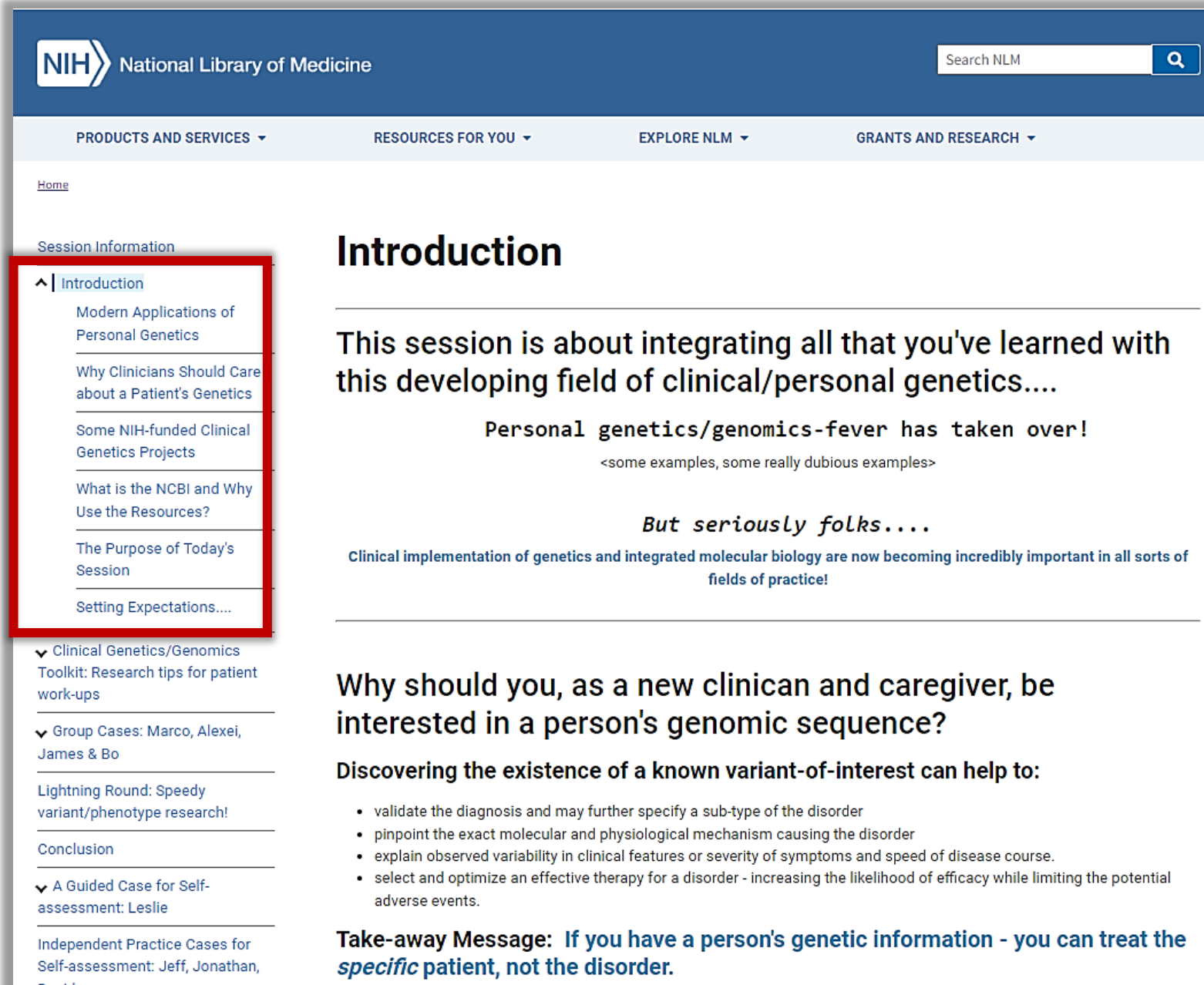
▶ **How should I prepare for this session?**

In Session 164:

- We will discuss the state of clinical practice with regard to the application of precision medicine principles.
- We will work together through a cluster of similar-sounding patient cases and discover the underlying cause of pathology in each of these real patients.
- By looking at the patient's diverse molecular mechanisms, we will explore how knowing the pathology of a patient's genetic disorder could be useful for precision diagnosis, explain the clinical presentation, and may be helpful in customizing the case management plan.

NCBI WORKSHOP

Introduction

Some background information &

setting up today's activity!

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Personal Genetics/Genomics fever
has taken over!

Wait! What?

Wide-spread application of genetic/genomic information started out with human migration & ancestry,

and from there, family history & phenotypes,

what type of diet and/or what you should eat,

and from there health, nutrition, personality, family relationships, response to cannabis
& DNA Artwork!

DYNAMIC DNA LABORATORIES

Order a Test ⌄   Bundles ⌄   About ⌄   Local COVID-19 Testing   Account   Register a Kit

🧬 Personalized Vitamins are here! Log in to view your formula based on your Nutrition DNA test.

Home Health Tests

Proactive Health   Family + Ancestry   Personalized Medicine   Substance   DNA Artwork

Personalized Medicine

Pain Management
PGx Test

- Reduce trial and error to 40+ medications
- Dosage recommendations
- Suggest alternatives to high-risk medications

Cardiovascular Health
PGx Test

- Eliminate trial and error for 30+ medications
- Dosage recommendations
- Find alternatives to high-risk medications.

Mental Health
PGx Test

- Eliminate trial and error to 90+ medications
- Dosage recommendations
- Find alternatives to high-risk medications

Comprehensive
PGx Test

- Eliminate trial and error for 150+ medications
- Dosage recommendations
- Find alternatives to high-risk medications

Proactive Health

Nutrition
DNA Test

- Optimal diet for weight loss
- Food reactions and nutritional needs
- Healthy eating behaviors

Fitness
DNA Test

- Optimal exercise for weight loss
- Muscle strength and growth
- Injury risk and recovery time

Skin
DNA Test

- Anti-aging and nutritional needs
- Predispositions to skin health risks
- Solutions for problematic skin

Personality
DNA Test

- Character insights
- Mood, behavior, and persona
- Mind-body connection

Family + Ancestry

Ancestry
DNA Test

- Maternal and paternal haplogroups
- Ancestral migration patterns
- Ethnic composition

Paternity
DNA Test

- Reliable paternity confirmation
- Fully confidential results
- Simple and easy cheek swab

Siblingship
DNA Test

- Confirmation of familial relationships
- Fully confidential results
- Easy to read report

Substance

Cannabis
DNA Test

- Discover your CBD and THC metabolism
- Potential side effects from cannabis use
- Dosing and product selection guidelines

DNA Artwork

DNA Portrait

$199   View

what sport/position you should play,

what wine you should buy….

VINOME

As of January 1, 2020 Vinome is taking a break.

All Helix customers will continue to have access to other applications that were purchased on the Helix marketplace, but Vinome results will not be accessable.

We want to thank all of our customers who took the journey into wine, guided by their DNA.

Your DNA Guide to Wines You'll Love

Take the guesswork out of buying wine. We analyze your DNA and taste preferences, then match you with hard-to-find wines selected for your unique palate. Shop for your bottles in our online store, or join our wine club. Either way, we deliver to your doorstep.

Vino + Genome = Vinome

SIGN UP

Have you.....

○ Thought about taking a genetic test, but have not
○ Taken a direct-to-consumer genealogical/ancestry DNA test
○ Taken a direct-to-consumer genetic test with health-related information
○ Seen a genetic test for a patient
○ Seen a case management plan changed based on a genetic test result
○ Given your dog a genetic test

But seriously, folks…

Using Genetic/Genomics to assist in
High-Definition Diagnosis &
Precision Treatment Selection

High Definition & Precision?

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An outline of a history of medical practice

- The Art of Medicine

- The Science of Medicine

- Evidence-based Clinical Practice

  "Evidence-based medicine is the integration of best research evidence with clinical expertise and patient values."

- "Personalized" Medicine

  [AMA – all patient-care should be "personalized"]

- "Precision" Medicine

  "*Precision medicine* is an emerging approach for disease diagnosis treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person."

- *and now?*
  .....Molecular Medicine, Genomic Medicine, High-Definition Medicine....

HIGH-DEFINITION MEDICINE?

Establish an Individual's **Baseline** of Health

Create a Personalized **"Prevention" Strategy**

Perform **High-Definition Diagnosis** & Select **High-Precision Treatment**
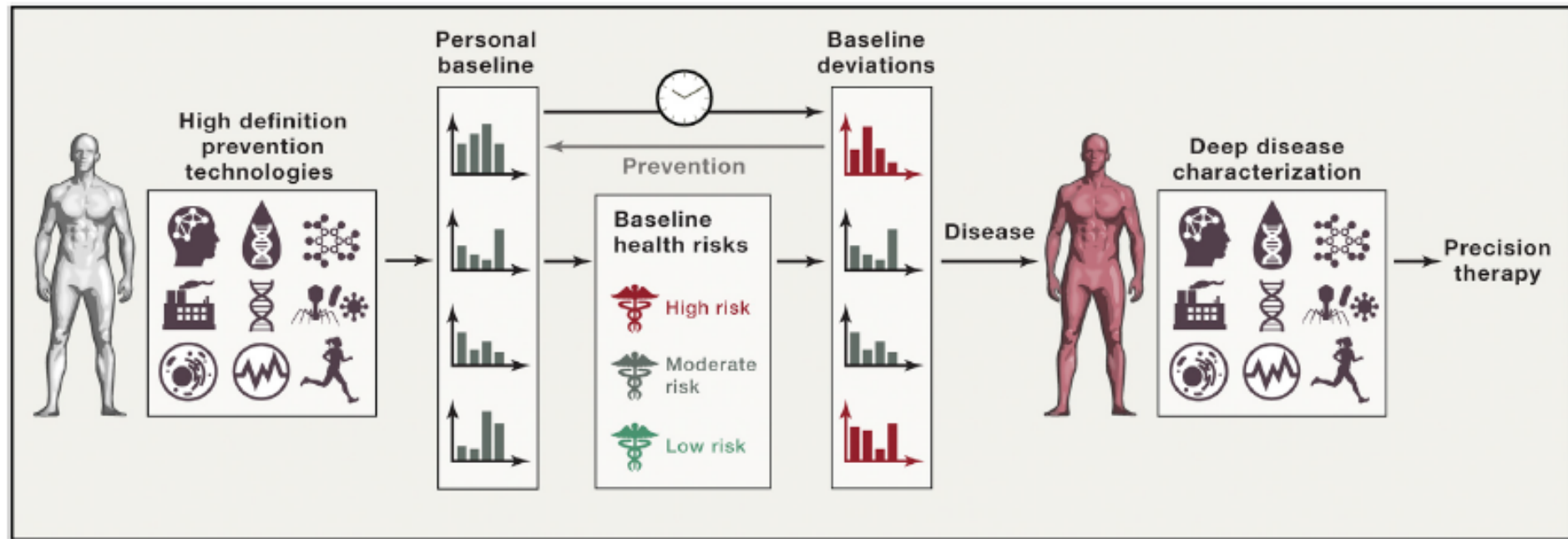
"High-Definition Medicine." Torkamani A, Andersen KG, Steinhubl SR, and EJ Topol.
Cell. 24 August 2017 170(5), 828–843.

"Precision" Medicine – NIH funded research projects
for disease characterization and to improve diagnoses & treatments
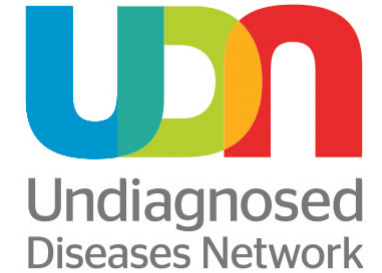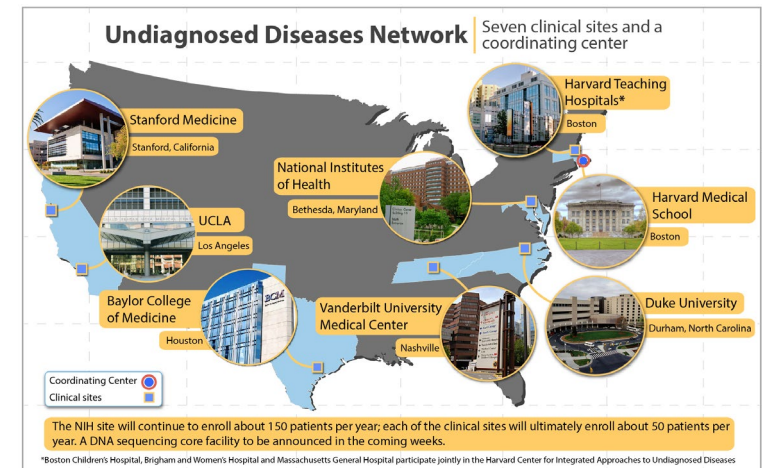
WHY "NCBI"?

National Center for Biotechnology Information
A Division of the U.S. National Library of Medicine

We are a "center" within the NLM responsible
for creation, curation and maintenance of
medical and scientific databases
and other things...

We receive, create, archive &
make available biomedical information,
as well as perform computational biology
& IT systems research....

MedGen

Genetic Testing Registry (GTR)

ClinicalTrials.gov

dbGaP

ClinVar

Genome   Assembly

dbSNP

dbVar

SRA

Nucleotide

Books

GeneReviews

Medical Genetics Summaries

PubMed

PubMed Central (PMC)

GEO

NLM Catalog (Journals)

BioSystems

Gene

PubChem Compound

PubChem BioAssay

Protein

Conserved Domains Database (CDD)

Structure
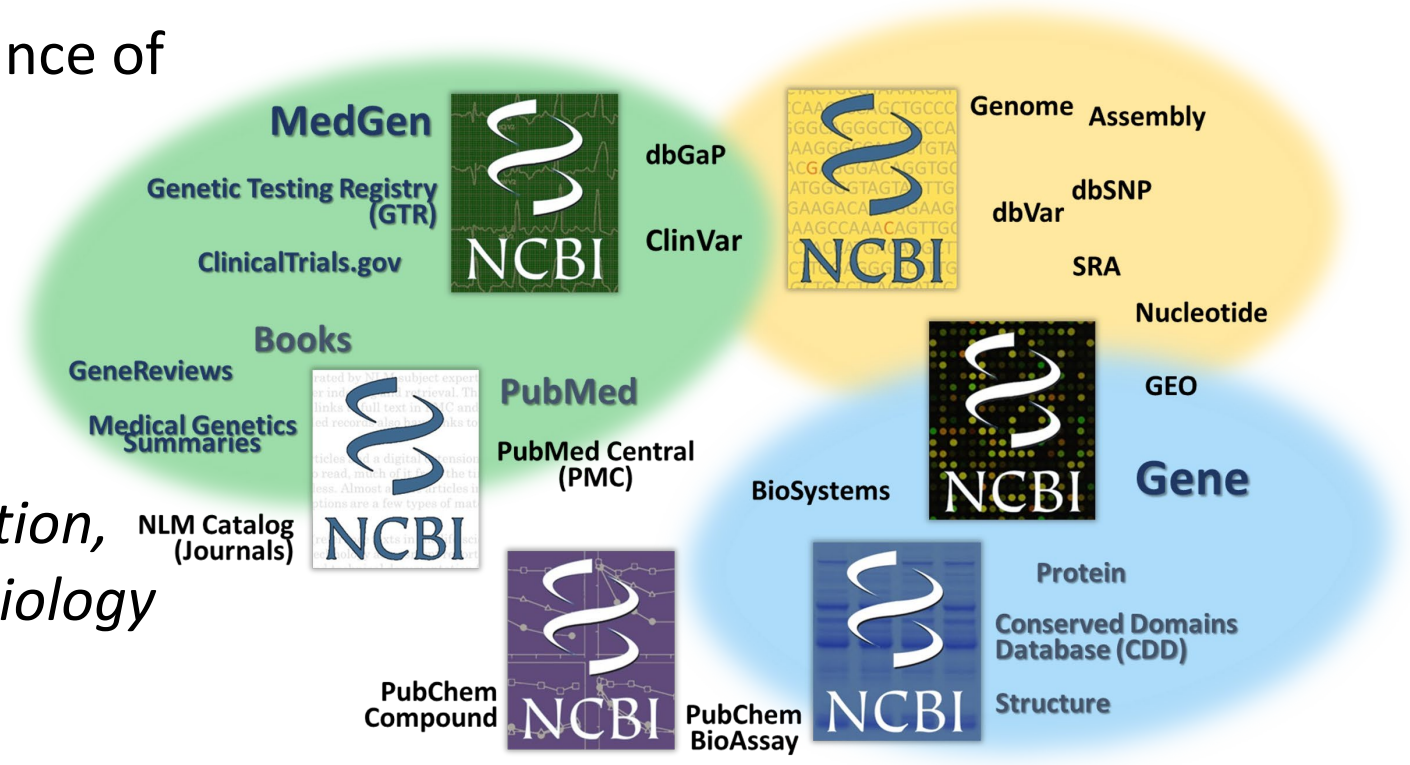
we aspire to help make sense of all that information!

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FOUNDATIONS SESSION 164

Molecular Pathology Case Studies:

Connecting the dots between

- *Genetics*
- *Molecular biology*
- *Biochemistry*

in real patients!

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 and has recently developed clinically-focused resources enabling scientists and clinicians to integrate known molecular biological information with clinically-relevant genetic variations.

Today:

- We will discuss the application of precision medicine principles in the clinic.

- We will work together through a cluster of similar-sounding patient cases and discover the underlying cause of pathology in each of these real patients.

- By looking at the diverse molecular mechanisms seen in these patients, we will:
  - explore how knowing the pathology of a patient's genetic disorder can facilitate a "precision diagnosis"
  - explain the clinical presentation
  - and can be helpful in customizing the case management plan.

- There are some additional cases for you to work on for practice & self-assessment.

**This session was designed to help you
review the major concepts you've been learning**

& to give you experience in integrating all of them in real-world patient case studies!

SETTING EXPECTATIONS

What we will cover

- NCBI's web-based **resources** that may be helpful to learn about human genetic disorders/conditions, genetic variations, genes and gene products.

- **Examples** of how you can use these resources from real clinical cases to explore and understand the underlying molecular pathology.

What we will *not* cover

- basic genetics or genetic principles - although we *will* use your knowledge about these in our discussions.

- high-throughput variant analysis - but you may find some helpful resources in the provided reference materials.

- resources for *all* human disorders, today we are focusing on simple genetic disorders. (for example, we will not discuss polygenic disorders such as most cancers or variants in disease-causing pathogens)

- Today's workshop is about *finding helpful information* you can use in your research.
  We will not discuss best practices for implementation of genetic information in the clinic or production of diagnostic or therapeutic products.

Disclaimer

⇒ We cannot make recommendations on what you should do.
   We provide resources, data and information that you can use as part of your work.

Let's get this out of the way – *"How am I going to be tested on this?"*

You are going to be tested on this in several ways:

Next week's exam

- *You will **not** have to run through a whole case study.*
- *You will **not** be tested on the specifics of each of these cases.*
- **You will need to be able to answer questions based on *application* of genetics/molbiol/biochem in patient cases:**
  - When is genetic testing something to consider and how does it fit within clinical cases?
  - Why is it helpful to integrate molecular sciences for decisions about patient care & case management?
  - How are problems at the molecular level (integrated molecular pathology) related to health?
  - Where can you find high-quality biological information *when you need it?*

USMLE Step 1

- Integration of molecular sciences information will help you answer case study-driven questions.
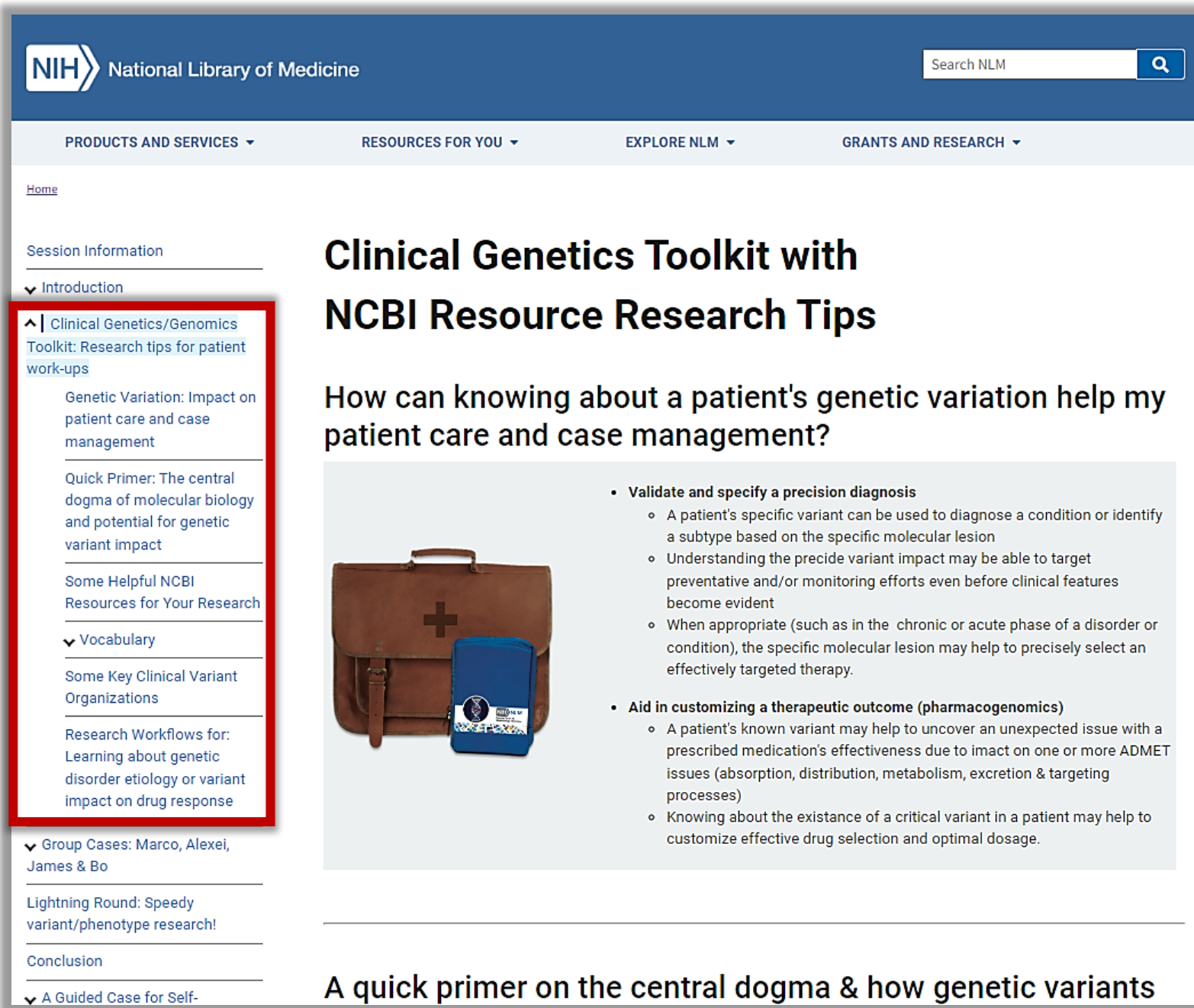
Your CAREER!

- Keep up with science, technology with an increasingly-specific focus on individual patient's cases
- Resident Preceptors, Attending Physicians, etc. – will all be asking you questions…..
- Patients & Parents increasingly wanting to know "why?" Be able to fully explain things!

Optional Worksheet: *a WordDoc to fill out*

Group Cases

Marco   Alexei   James   Bo

REFERRAL   TEST RESULTS (for each boy)

A **Case Workup worksheet** for use to keep track of *your* patient's information.

OR, if you prefer, here is a **Group Case Workup worksheet** to track them all at in one place.

Today, you are going to work with one of these boys!

Your Patient's Info	NOTES
Phenotype	
Preliminary Diagnosis	
Genetic Variation(s)	
Laboratory Assertion(s)	
Variant Information: • Asserted interpretation listed in **ClinVar** • HGVS names from **ClinVar** • Is population data available in **dbSNP**?	
Gene Information in NCBI Gene: • Symbol and Name • Gene Summary • Tissue Expression information • Gene Ontology information	
Ultimate Impacted Biomolecule based on: • **GDV** to view the chromosome and gene region • **RefSeqGene Graphics** view of gene region and transcript(s) • **RefSeq Protein Graphics** view of protein and domains • **CDD or iCn3D** to view a structure, *as needed*	
Proposed Molecular Mechanism of Variant Impact	
How does this relate back to the phenotype (symptoms/clinical features & diagnosis)?	
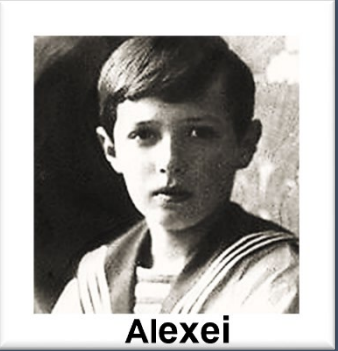
Researching the Referral

Read the **Referral** – *and take notes!*

	Marco	Alexei	James	Bo
Phenotype (including severity)				
Preliminary Diagnosis				

If you want to learn more about this disorder…..Search in **MedGen**!

Read the **Referral** – *and take notes!*

	Marco	**Alexei**	**James**	**Bo**
Phenotype (including severity)	significant bruising and severe pain after his first soccer practice previous episodes of scratches causing prolonged bleeding family history: a male cousin was known to have hemophilia.	severe hematoma on thigh after bumping into a boat's oarlock. long history of recurrent episodes of illness (bruises, bleeding episodes, and long painful recoveries) since shortly after birth Family history: • rumors of bleeding issues in many cousins of the maternal family.	relentless nosebleed caused by "bumping into a coffee table" visible bruising on his knees and palms since he began crawling at 6 months Family history: • Mother required a blood transfusion after natural childbirth • Maternal uncle died at the age of 6 years old from a "brain bleed" after a fall.	profuse bleeding laceration on left index finger previous episodes of prolonged bleeding which hadn't "risen to the level of an ER visit but were concerning." Family history: • No "genetic" family history is available as Bo was adopted from China at the age of 3 years old
Preliminary Diagnosis	Hemophilia *(sub-type not determined yet)*	Hemophilia *(sub-type not determined yet)*	Hemophilia *(sub-type not determined yet)*	Hemophilia *(sub-type not determined yet)*

To learn more about this disorder…..Search in MedGen! NCBI WORKSHOP

Edward
Duke of Kent

Victoria
Princess of Saxe-Coburg
(1786-1861)

Albert

Victoria
Queen of England

Normal Male — Hemophilic Male
Normal Female — Carrier Female

Victoria — Frederick — Edward VII of England — Alexandra

Alice — Louis of Hesse — Alfred — Helena — Louise — Arthur — Leopold — Helen — Beatrice — Henry

Wilhelm II — Sophie of Greece — George V

Irene — Henry — Fred — Alix — Nikolas II of Russia

Alice of Athlone

Alfonso XIII of Spain — Eugenie — Leopold — Maurice

George VI

Waldemar — Prince Sigmund of Prussia — Henry — Olga — Tatiana — Marie — Anastasia — Alexis

Lady May Abel Smith — Rupert — ?

Alfonso — Gonzalo

Princess Margaret — Queen Elizabeth — Prince Phillip

Princess Diana — Prince Charles — Princess Ann — Prince Andrew — Prince Edward

Juan Carlos of Spain

Duchess Meghan — Prince Harry — Prince William — Princess Catherine

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NCBI WORKSHOP

Understanding the Genetic Test Results

Open your **Genetic Test Report**
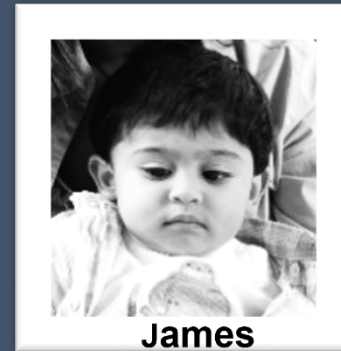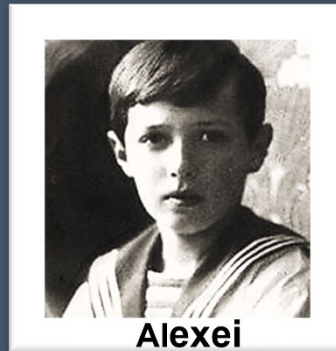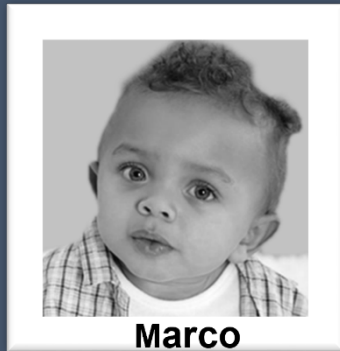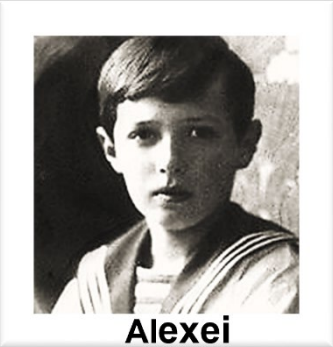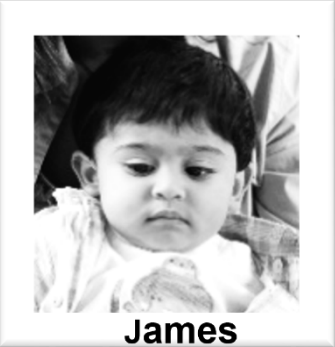
	Marco	Alexei	James	Bo
Genetic Variation(s)				
Laboratory Assertion(s)				

Open your **Genetic Test Report**

	Marco	Alexei	James	Bo
Genetic Variation(s)	NG_011403.1: g.4980_5005del	F9 c.278-3A>G	F8 p.Arg15Ter	F9 p.Asp110Gly
Laboratory Assertion(s)	variant of uncertain significance (VUS)	pathogenic	pathogenic	pathogenic

Learning about the identified variant

Search ClinVar with the Genetic Test Reported Variant

Genetic Variation(s)	Marco NG_011403.1: g.4980_5005del	Alexei F9 c.278-3A>G	James F8 p.Arg15Ter	Bo F9 p.Asp110Gly
Variant Information:				
• **Asserted interpretation in ClinVar** • **HGVS names from ClinVar**				
• **Is population data available in dbSNP?**				

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Marco's genetic variant isn't showing up in ClinVar. Why?
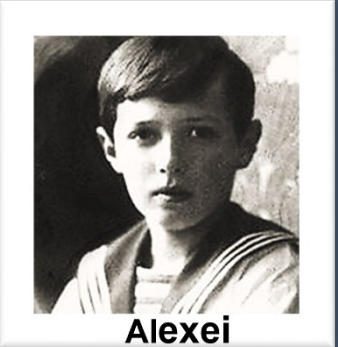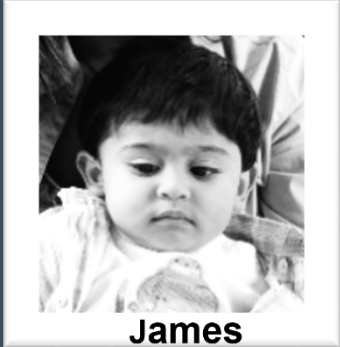
A. Because I typed it in wrong.

B. Because ClinVar's search isn't working.

C. Because no one has ever found that variant before.

D. Because no one in a clinical or research lab has submitted information about that particular variant.

Search **ClinVar** with the Genetic Test Reported Variant
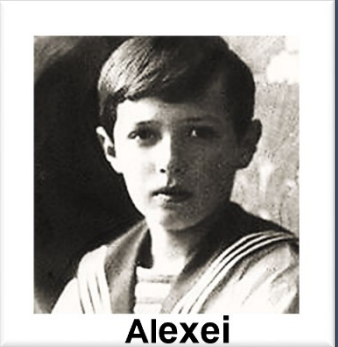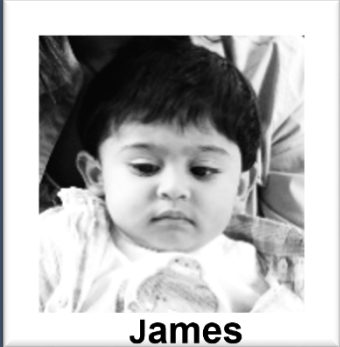
	Marco	Alexei	James	Bo
Genetic Variation(s)	**NG_011403.1:** **g.4980_5005del**	**F9 c.278-3A>G**	**F8 p.Arg15Ter**	**F9 p.Asp110Gly**
Variant Information: • **Asserted interpretation in ClinVar** • **HGVS names from ClinVar**	**not in ClinVar!** *(assumed VUS)*	**pathogenic**	**pathogenic**	**pathogenic**
	NG_011403.1(F8): **g.4980_5005del** **Note:** no protein HGVS	**NG_007994.1(F9):** **g.15338A>G** **Note:** no protein HGVS	**NG_011403.2(F8):** **g.5214C>T** **NP_000123.1(F8):** **p.Arg15Ter**	**NG_007994.1(F9):** **g.15392A>G** **NP_000124.1(F9):** **p.Asp110Gly**
• **Is population data available in dbSNP?**	Not in Clinvar. Searching directly in dbSNP did not find anything.	rs398122990 Yes! And it is really, really rare.	rs387906432 Yes! And it is really, really rare.	rs137852234 Yes! And it is pretty darn rate.

Learning about the implicated gene
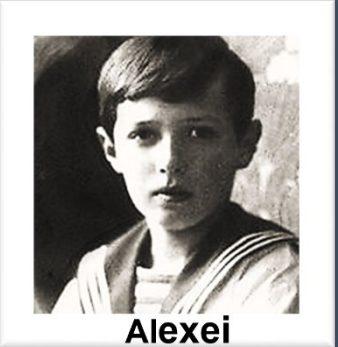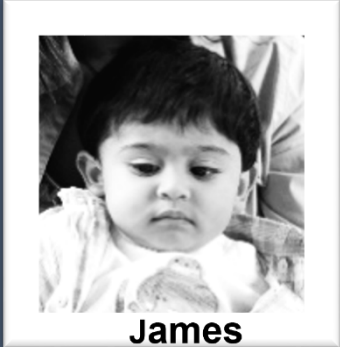
Search **Gene** with the Test Reported Gene Symbol

	Marco	Alexei	James	Bo
Gene Symbol	F8	F9	F8	F9
Gene Information in NCBI Gene:				
• **Gene Name** • **Gene Summary**				
• **Tissue Expression information**				
• **Gene Ontology information**				

Search **Gene** with the Test Reported Gene Symbol

	Marco	Alexei	James	Bo
Gene Symbol	**F8**	**F9**	**F8**	**F9**
Gene Information in NCBI Gene: • **Gene Name** • **Gene Summary** • **Tissue Expression information** • **Gene Ontology information**	Coagulation factor VIII	Coagulation factor IX	Coagulation factor VIII	Coagulation factor IX
	...participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa. ....Defects in this gene results in hemophilia A, a common recessive X-linked coagulation disorder. [provided by RefSeq, Jul 2008]		...participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa. ....Defects in this gene results in hemophilia A, a common recessive X-linked coagulation disorder. [provided by RefSeq, Jul 2008]	
	Broad expression, especially in Liver, Spleen and others		Broad expression, especially in Liver, Spleen and others	
	Extracellular Blood coagulation Protein binding		Extracellular Blood coagulation Protein binding	

Search **Gene** with the Test Reported Gene Symbol

	Marco	Alexei	James	Bo
Gene Symbol	F8	F9	F8	F9
	Coagulation factor VIII	Coagulation factor IX	Coagulation factor VIII	Coagulation factor IX
Gene Information in NCBI Gene: • **Gene Name** • **Gene Summary** • **Tissue Expression information** • **Gene Ontology information**		…vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen….converted to an active form by factor XIa, …activates factor X … through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene…cause factor IX deficiency, which is a recessive X-linked disorder….[provided by RefSeq, Sep 2015]		…vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen….converted to an active form by factor XIa, …activates factor X … through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene…cause factor IX deficiency, which is a recessive X-linked disorder….[provided by RefSeq, Sep 2015]
		Pretty much just expressed in the liver		Pretty much just expressed in the liver
		Extracellular Blood coagulation Ca+2-binding & endopeptidase		Extracellular Blood coagulation Ca+2-binding & endopeptidase

Search **Gene** with the Test Reported Gene Symbol

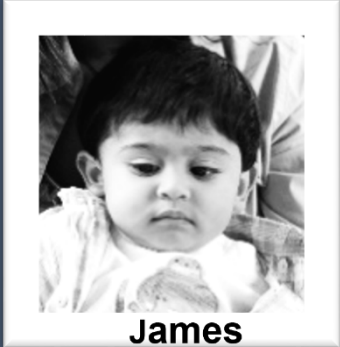| Gene Symbol	Marco	Alexei	James	Bo
	F8	**F9**	**F8**	**F9**
Gene Information in NCBI Gene: • **Gene Name** • **Gene Summary** • **Tissue Expression information** • **Gene Ontology information**	**Coagulation factor VIII**	**Coagulation factor IX**	**Coagulation factor VIII**	**Coagulation factor IX**
	…participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa. ….Defects in this gene results in hemophilia A, a common recessive X-linked coagulation disorder. [provided by RefSeq, Jul 2008]	…vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen….converted to an active form by factor XIa, …activates factor X … through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene…cause factor IX deficiency, which is a recessive X-linked disorder….[provided by RefSeq, Sep 2015]	…participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca+2 and phospholipids, converts factor X to the activated form Xa. ….Defects in this gene results in hemophilia A, a common recessive X-linked coagulation disorder. [provided by RefSeq, Jul 2008]	…vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen….converted to an active form by factor XIa, …activates factor X … through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene…cause factor IX deficiency, which is a recessive X-linked disorder….[provided by RefSeq, Sep 2015]
	Broad expression, especially in Liver, Spleen and others	Pretty much just expressed in the liver	Broad expression, especially in Liver, Spleen and others	Pretty much just expressed in the liver
	Extracellular Blood coagulation Protein binding	Extracellular Blood coagulation Ca+2-binding & endopeptidase	Extracellular Blood coagulation Protein binding	Extracellular Blood coagulation Ca+2-binding & endopeptidase

About the clotting cascade

Factor 8 (F8 a.k.a. FVIII)

& Factor 9 (F9 a.k.a. FIX)

are critical regulatory factors for blood coagulation/clotting.

They also both happen to be encoded on the X-chromosome.

Males only have 1 copy of the gene and therefore are dramatically impacted if there is a pathogenic variant impacting even just one of these two genes.

INTRINSIC PATHWAY

COLLAGEN
KALLIKREIN
HMWK

(HAGEMAN FACTOR) XII → XIIa

C XI → XIa
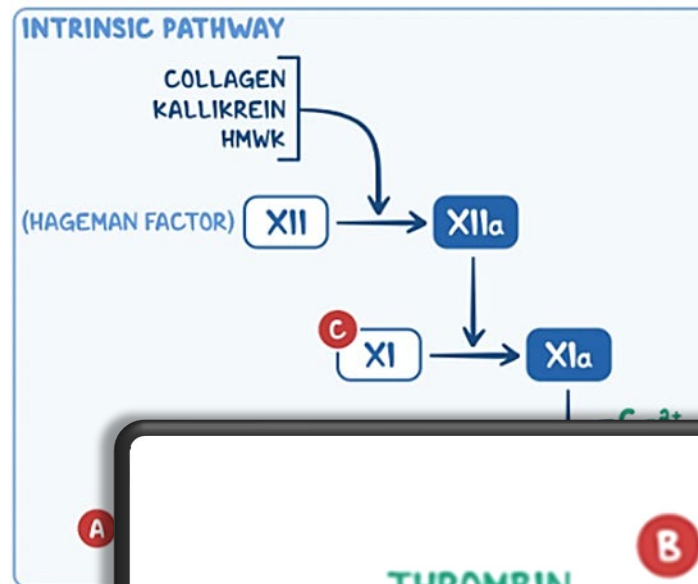
BACKGROUND

* SERIES of STEPS in RESPONSE to BLEEDING CAUSED by TISSUE INJURY
  ~ EACH STEP ACTIVATES the NEXT & ULTIMATELY PRODUCES a BLOOD CLOT
* aka SECONDARY HEMOSTASIS

EXTRINSIC PATHWAY

DAMAGE to ENDOTHELIAL TISSUE

Ca^{2+}

B E IX → IXa

A D VIII → THROMBIN → VIIIa

Ca^{2+}

COMMON PATHWAY

THROMBIN

E X → Xa

(STABILIZING FACTOR) XIII → XIIIa

Ca^{2+}

STABLE FIBRIN CLOT
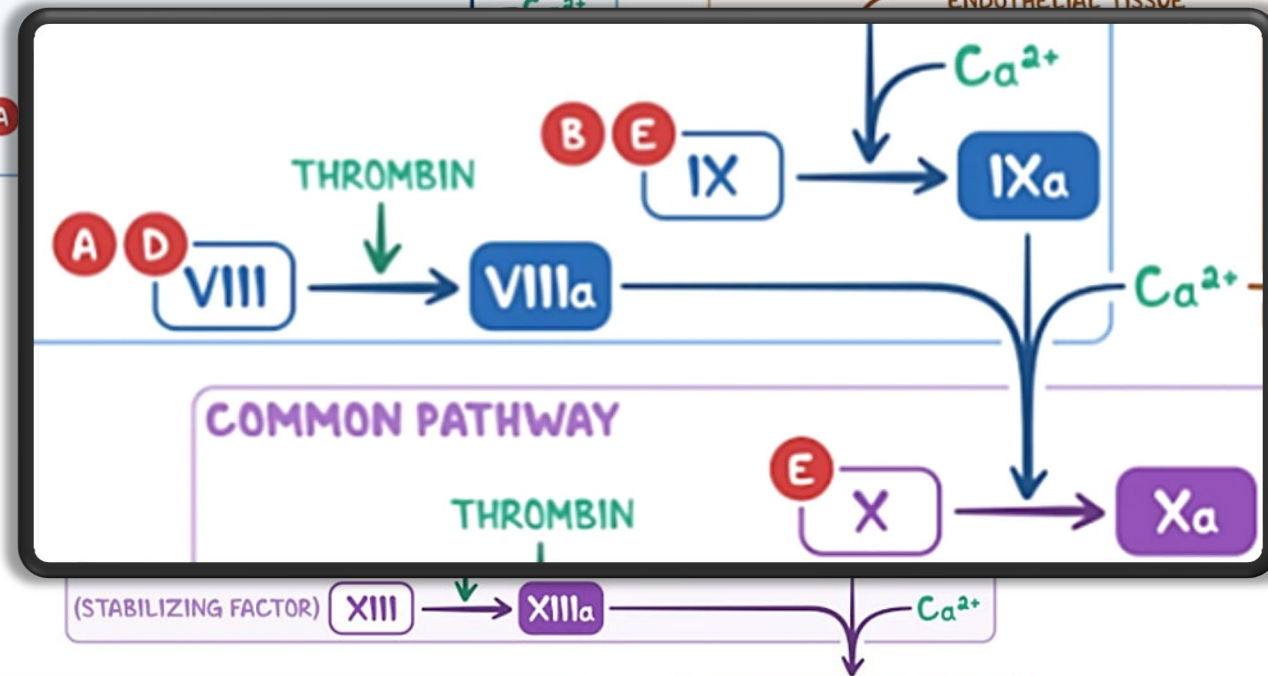
COAGULATION DISORDERS

* CAN EITHER CAUSE EXCESSIVE or INADEQUATE CLOTTING
* DEFICIENCY in ≥1 CLOTTING FACTOR

A HEMOPHILIA A   D von WILLEBRAND DISEASE
B HEMOPHILIA B   E VITAMIN K DEFICIENCY
C HEMOPHILIA C

https://www.osmosis.org/answers/coagulation-cascade

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Mapping the variant

Map the Variant with Genome/Sequence Browser Tools

Genetic Variation(s)	Marco	Alexei	James	Bo
	NG_011403.1(F8):g.4980_5005del	NG_007994.1(F9):g.15338A>G	NG_011403.2(F8):g.5214C>T	NG_007994.1(F9):g.15392A>G
		NP_000124.1(F9):p.Gly94Ter	NP_000123.1(F8):p.Arg15Ter	NP_000124.1(F9):p.Asp110Gly
Ultimate Impacted Biomolecule based on:				
• **GDV** to view the chromosome and gene region				
• **RefSeqGene Graphics view** of gene region and transcript(s)				
• **RefSeq Protein Graphics view** of protein and domains				
• **CDD or iCn3D** to view a structure				

An overview of the central dogma & locating a genetic variation
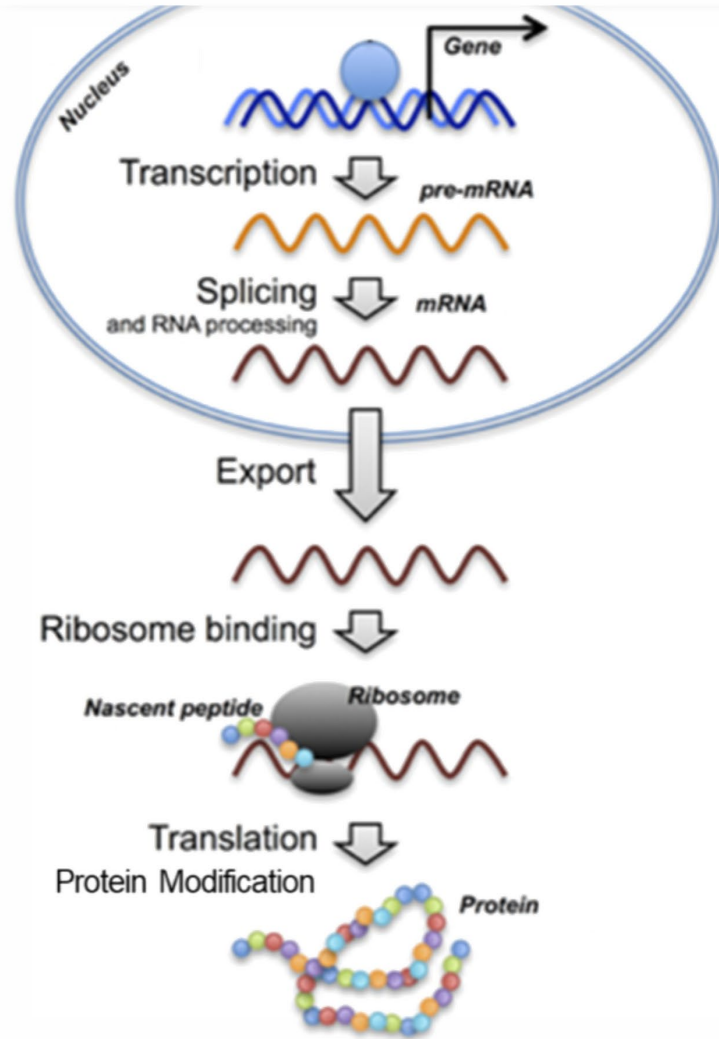
An overview of the central dogma & locating a genetic variation

Is the variant in the:

☑

☐

☐

☐

☐

Genome, assembly level → GRCh38

Chromosome/DNA → NC_ (RefSeq)

> **HGVS Variant Example: NC_000002.12:g.72888457A>G**
> *might impact chromatin structure or function*

Gene/DNA, gene *region* level → NG_*(RefSeqGene)

> **HGVS Variant Example: NG_008234.1(SPR):g.6075A>G**
> *might impact expression for or splicing of a gene transcript*

Transcript/RNA → NM_ , NR_ ("RefSeq RNA"|RefSeq Select)

> **HGVS Variant Example: NM_003124.5(SPR):c.448A>G**
> *might impact transcript processing and/or translation*

Protein, mRNA-encoded sequence → NP_*("RefSeq Protein")

> **HGVS Variant Example: NP_003115.1(SPR):p.Arg150Gly**
> *might impact protein folding, processing or function*

Structure

Map the Variant with **Genome/Sequence Browser Tools**

	Marco	Alexei	James	Bo
Genetic Variation(s)	NG_011403.1(F8):g.4980_5005del	NG_007994.1(F9):g.15338A>G NP_000124.1(F9):p.Gly94Ter	NG_011403.2(F8):g.5214C>T NP_000123.1(F8):p.Arg15Ter	NG_007994.1(F9):g.15392A>G NP_000124.1(F9):p.Asp110Gly
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Map the Variant with Genome/Sequence Browser Tools

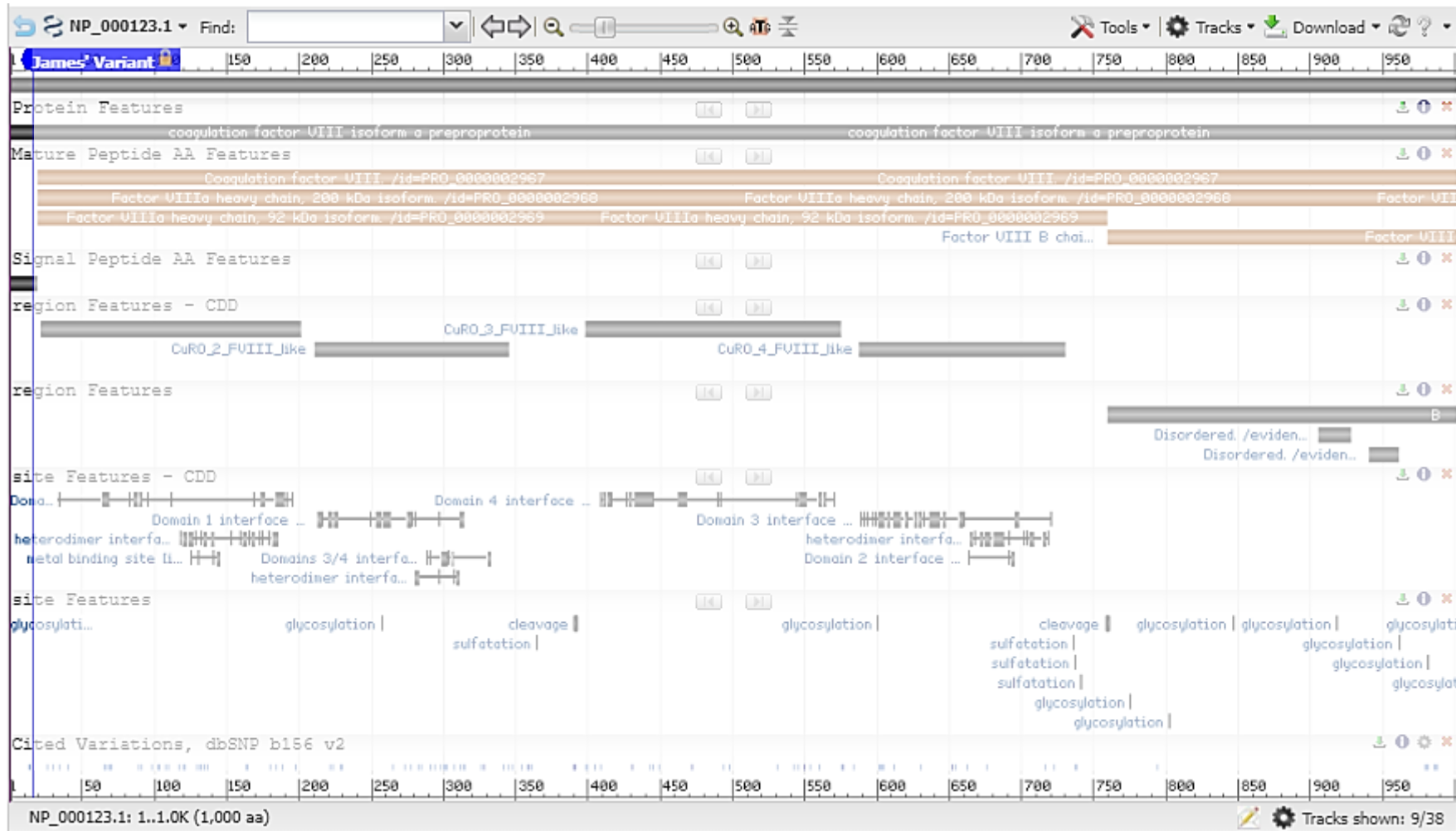|	Marco	Alexei	James	Bo
Genetic Variation(s)	NG_011403.1(F8):g.4980_5005del	NG_007994.1(F9):g.15338A>G NP_000124.1(F9):p.Gly94Ter	NG_011403.2(F8):g.5214C>T NP_000123.1(F8):p.Arg15Ter	NG_007994.1(F9):g.15392A>G NP_000124.1(F9):p.Asp110Gly
Ultimate Impacted Biomolecule based on: • **GDV** to view the chromosome and gene region • **RefSeqGene Graphics view** of gene region and transcript(s) • **RefSeq Protein Graphics view** of protein and domains • **CDD or iCn3D** to view a structure	Deleted region upstream from through the beginning of the transcription start.		Located in the coding region within exon 1.	
	Transcripts are not expressed, therefore the variant does not impact biomolecules beyond the gene region in the chromosome.		Located within the first coding exon.	
			The coding sequence quickly terminates after only 14 residues – producing a non-functional peptide destined for degradation.	
			Most of the protein is never made so it cannot serve as a complex anchor for clotting factor aggregation.	

Marco's variant is a deletion of the region including the transcript start codon – so no transcript is produced.
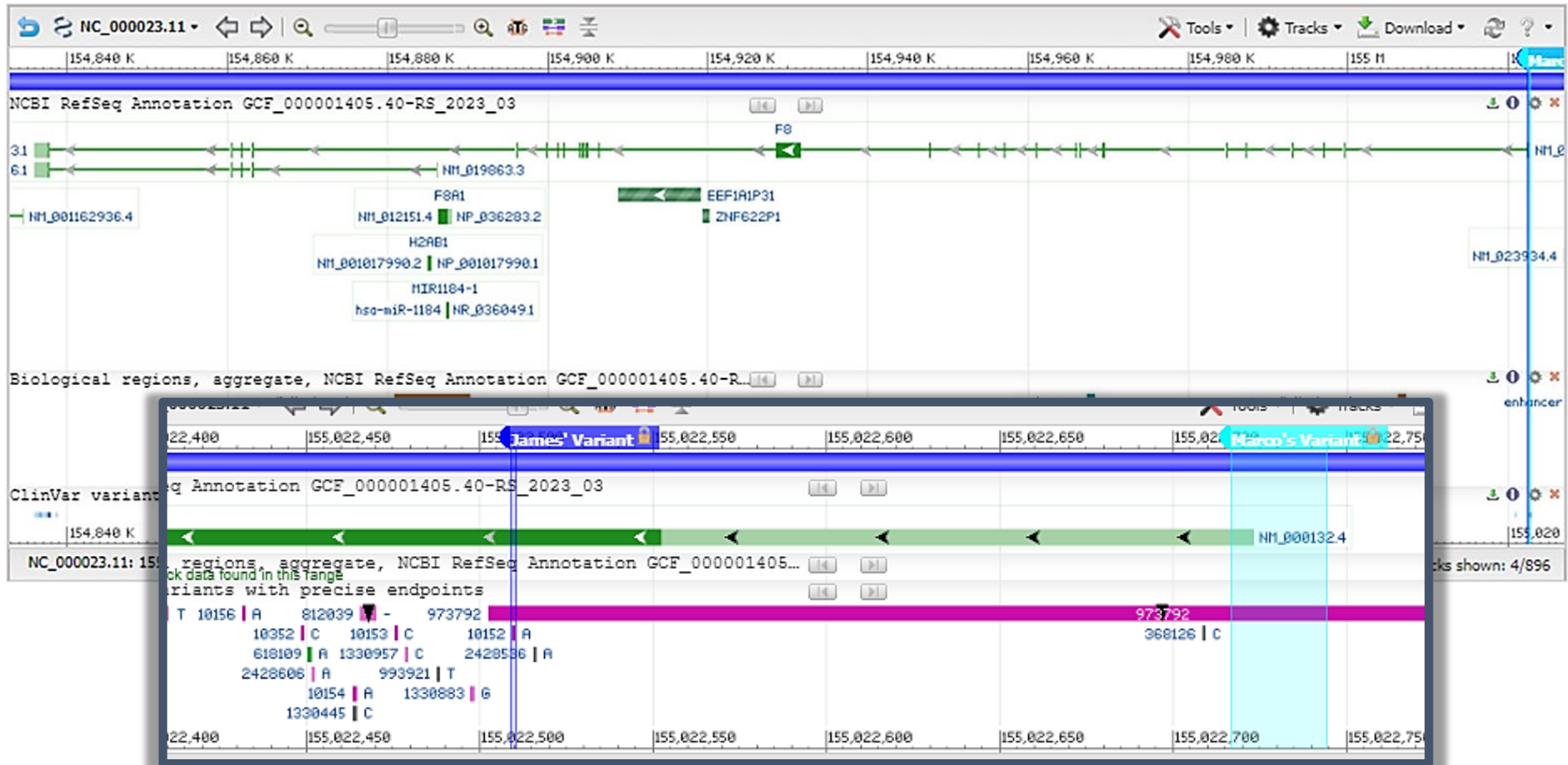
James' variant is in the coding region of the transcript – thus directly impacts the protein sequence.

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James' variant in the coding region of the transcript is a termination codon.


Thus, the full-length peptide is never made – actually most of it is gone!....so there is no functional protein made.
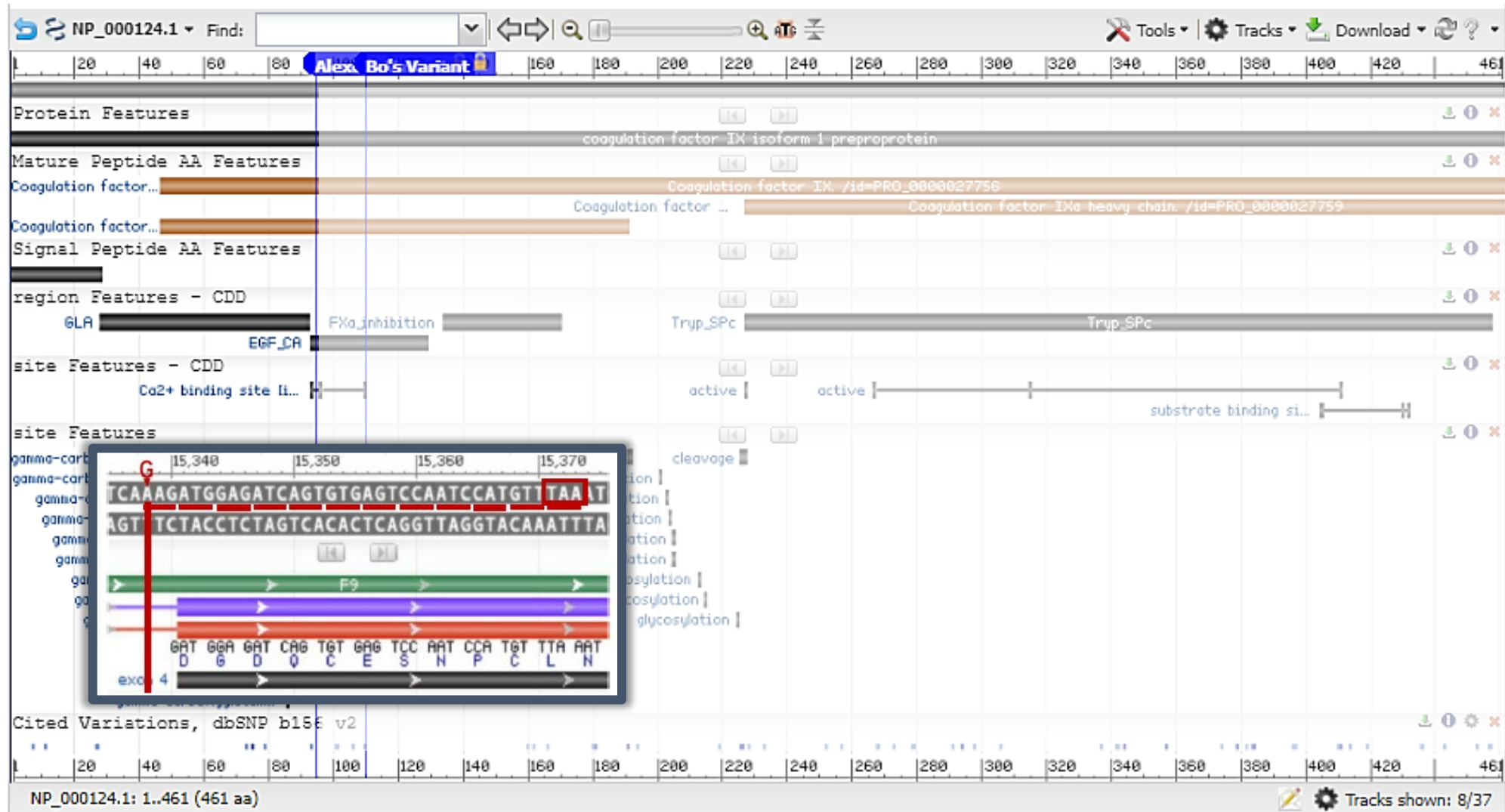
Map the Variant with **Genome/Sequence Browser Tools**

	Marco	Alexei	James	Bo
Genetic Variation(s)	NG_011403.1(F8):g.4980_5005del	NG_007994.1(F9):g.15338A>G **NP_000124.1(F9):p.Gly94Ter**	NG_011403.2(F8):g.5214C>T NP_000123.1(F8):p.Arg15Ter	NG_007994.1(F9):g.15392A>G NP_000124.1(F9):p.Asp110Gly
Ultimate Impacted Biomolecule based on: • **GDV** to view the chromosome and gene region		Located near a splice site in the gene just before exon 4.		Located in the coding region within exon 4.
• **RefSeqGene Graphics view** of gene region and transcript(s)		Exon 4's acceptor site is shifted back due to the variation – causing a frameshift of the coding sequence.		Located within the coding region within exon 4.
• **RefSeq Protein Graphics view** of protein and domains		The coding sequence frameshift encodes an 11-residue peptide and then a stop codon - prematurely terminating the protein.		The protein is made, but with a change in amino acid 110 from an acidic Aspartate to a neutral Glycine.
• **CDD or iCn3D** to view a structure		A large portion of the protein is never made – especially the endopeptidase domain which is critical for activating FX – and propagating the clotting cascade.		The variant is one of 3 residues annotated as critical for binding to Ca^{+2}. The change from acidic Aspartate residue to neutral Glycine impacts its binding to the Ca^{+2} & protein folding, thus processing & activation.

Alexei's variant is near the end of a coding exon.
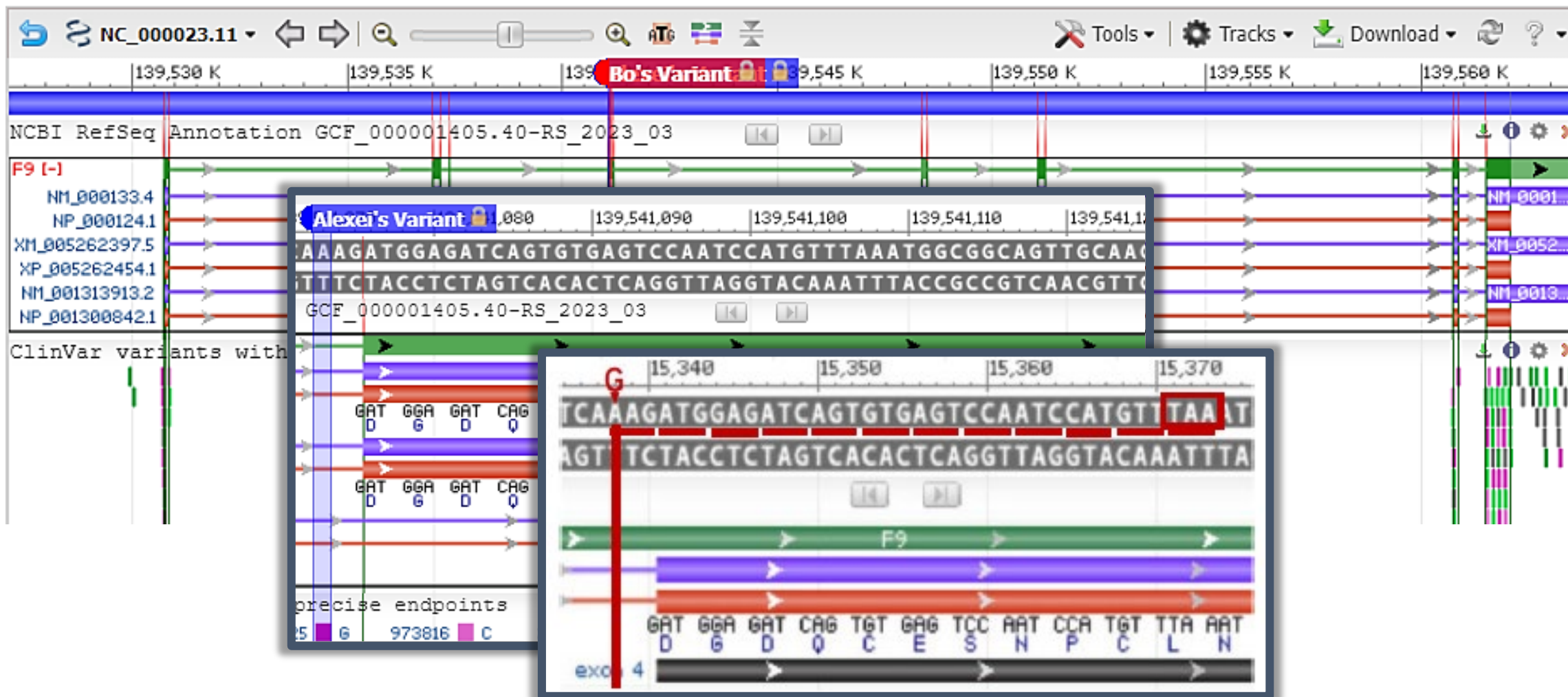
Because the variant creates an exon signal mimic, it shifts the exon splice site up two residues causing a frameshift in the protein coding sequence.

Alexei's splicing-shift and coding frameshift variant causes a short non-sense peptide with a termination codon.

**Thus, only the beginning of the peptide is made....so no functional protein is created.**

This is similar to what is seen for James' F8 protein.

Bo's variant is in the transcript's protein coding region and ends up in the protein sequence & structure!

The variant replaces one of the critical binding residues for the Calcium ion binding site – disrupting its ability to maintain a folded structure.

Thus, the protein isn't processed effectively leading to a smaller amount of active enzyme for the clotting cascade.

Map the Variant with **Genome/Sequence Browser Tools**

	Marco	Alexei	James	Bo
Genetic Variation(s)	NG_011403.1(F8):g.4980_5005del	NG_007994.1(F9):g.15338A>G **NP_000124.1(F9):p.Gly94Ter**	NG_011403.2(F8):g.5214C>T NP_000123.1(F8):p.Arg15Ter	NG_007994.1(F9):g.15392A>G NP_000124.1(F9):p.Asp110Gly
Ultimate Impacted Biomolecule based on: • **GDV** to view the chromosome and gene region • **RefSeqGene Graphics view** of gene region and transcript(s) • **RefSeq Protein Graphics view** of protein and domains • **CDD or iCn3D** to view a structure	Deleted region upstream from through the beginning of the transcription start.	Located near a splice site in the gene just before exon 4.	Located in the coding region within exon 1.	Located in the coding region within exon 4.
	Transcripts are not expressed, therefore the variant does not impact biomolecules beyond the gene region in the chromosome.	Exon 4's acceptor site is shifted back due to the variation – causing a frameshift of the coding sequence.	Located within the first coding exon.	Located within the coding region within exon 4.
		The coding sequence frameshift encodes an 11-residue peptide and then a stop codon - prematurely terminating the protein.	The coding sequence quickly terminates after only 14 residues – producing a non-functional peptide destined for degradation.	The protein is made, but with a change in amino acid 110 from an acidic Aspartate to a neutral Glycine.
		A large portion of the protein is never made – especially the endopeptidase domain which is critical for activating FX – and propagating the clotting cascade.	Most of the protein is never made so it cannot serve as a complex anchor for clotting factor aggregation.	The variant is one of 3 residues annotated as critical for binding to Ca^{+2}. The change from acidic Aspartate residue to neutral Glycine impacts its binding to the Ca^{+2} & protein folding, thus processing & activation.

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Putting it all together
tell the story….

Put it all together…
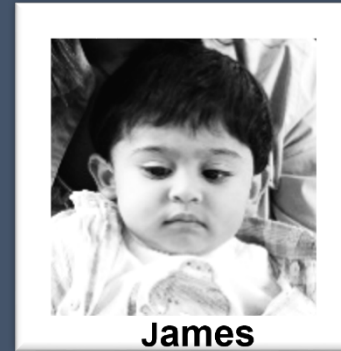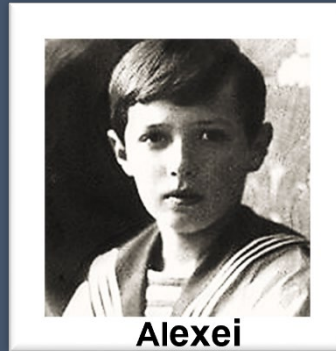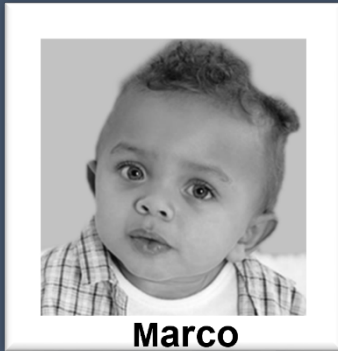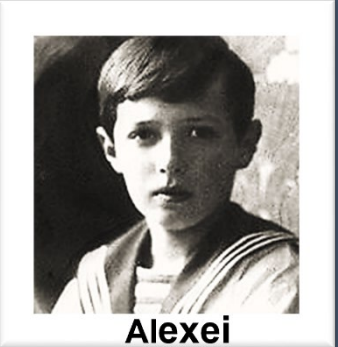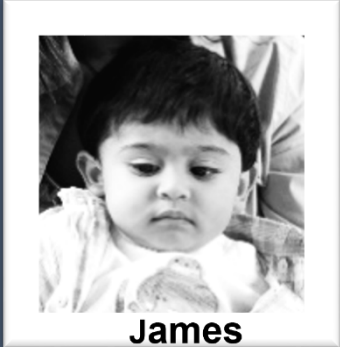
	Marco	Alexei	James	Bo
Diagnosis				
Genetic Variation(s)				
Proposed Molecular Mechanism of Variant Impact				
How does this relate back to the phenotype (symptoms/ clinical features & diagnosis)?				

Put it all together…

	Marco	Alexei	James	Bo
Diagnosis	Hemophilia A	Hemophilia B	Hemophilia A	Hemophilia B
Genetic Variation(s)	NG_011403.1(F8): g.4980_5005del	NG_007994.1(F9): g.15338A>Gr	NP_000123.1(F8): p.Arg15Ter	NP_000124.1(F9): p.Asp110Gly
Proposed Molecular Mechanism of Variant Impact	The deletion in the region just upstream and after the transcriptional start site - likely removes promoter elements and does not allow for gene expression.	This is a change in a splice site base – shifting the splicing back two positions, causing a coding frameshift and ending in premature termination.	This changes an amino acid coding codon to a premature termination codon.	This changes an acidic residue which is needed for binding a critical calcium ion which is required for F9 function.
How does this relate back to the phenotype (symptoms/clinical features & diagnosis)?	With a non-expressible F8, the clotting cascade will not be able to progress to create clots. This correlates with a severe phenotype.	With an F9 protein prematurely terminated, the catalytic domain for activating the next clotting factor is not made and the clotting cascade cannot progress to create clots. This correlates with a severe phenotype.	With an F8 protein prematurely terminated, the binding domains for activating the next clotting factor is not made and the clotting cascade cannot progress to create clots. This correlates with a severe phenotype.	With the loss of one of 3 coordinating residues for a critical calcium ion, the F9 protein is not fully functional and may not effectively activate the next clotting factor. This correlates with a less severe phenotype.

Leslie's Case
- *your workflow practice* -

A guided case to practice on your own!

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Guided Practice Case: Leslie

Welcome to Your Patient!

Leslie, a 40-year-old mother of two, was referred for genetic testing by her gastroenterologist...

Key Symptoms: Changes in bathroom habits, bleeding and abdominal pain

She has a medical history of colonic polyps. Her family history reveals that her maternal grandmother, mother and uncle had several forms of cancers including colon, breast, and endometrium. Her gastroenterologist suspects Lynch Syndrome.

QUESTION: What do you think is wrong with Leslie?

Researching the Referral

In this scenario, Leslie was referred to the genetics clinic because their preliminary diagnoses suggested that she might have a potentially serious genetic condition. If a known pathogenic genetic variant is found, it can validate the diagnosis and provide additional patient-specific information that might help customize her case management plan.

To learn more about a case, please **click on the Referral icon above** to open the form. Read it over and fill in below what you can glean from the description and proposed preliminary diagnosis.

	Notes
Phenotype	
Preliminary Diagnosis	

Independent Cases
- your workflow practice -

In your career you are going to get a patient history and/or referral information and potentially a genetic test result.

You won't get guided research steps.

So, here are three practice cases that you can try out!

But what if I'm in the clinic and *don't have time* to spend getting critical information?

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Important Concepts
- *to know* -

- Why should I care about molecular science while preparing to be a physician?

  knowledge is more power to act & act more effectively!

- How could understanding a patient's specific molecular pathology help you as a doctor?

  aiding in precision diagnosis,
  implementation of proactive/preventative measures
  including optimizing therapeutic selection,
  communicating with the patient & patient's family

- What in the patient's case record could start you thinking about *ordering* a genetic test?

  known genetic disorder with or without family history
  (don't trust commercial tests for your diagnosis!)

- Which databases are good places to start to find helpful disease/condition, gene information, and to validate a lab's call about a pathogenic genetic variation?

  NCBI MedGen , NCBI Gene & NCBI ClinVar