Welcome to your Patient – Detailed Answer

Raven (23 y.o. female) and James (8 m.o. male) American Mother & Son of Native American descent were referred by a local clinic....

Diagnosis: Bleeding Disorder, likely Hemophilia – due to normal CBC and platelet levels

Symptoms: Significant Bruising, Relentless Nose-bleed, with a possible history of bleeding issues in maternal line

Raven has noticed frequent bruising on his knees, palms and lower arms since he began crawling two months ago. Last night he pulled himself up to stand with the coffee table and fell - hitting his nose and causing a protracted nosebleed.

In further discussions with Raven, she described having “heavy periods” her whole life (although she assumed it was normal and was too embarrassed to talk with anyone about it) and she also mentioned a “bleeding-issue” after natural childbirth which required a transfusion. Upon further questioning, she mentioned that two years ago her younger brother died after falling out of a tree due to severe bleeding.

You decide to check both the son and mother for the presence of a hereditary bleeding disorder.

Summary of Clinical, Genetic and Molecular Biology Research

James appears to be exhibiting early symptoms of Hemophilia, while his mother, Raven, has shown evidence of some bleeding issues.

James’ lab tests confirm a diagnosis of Hemophilia due to elevated INR and APTT values with normal platelet levels. Since normal levels of Factor VIII activity and von Willebrand Factor were detected, this decreases the likelihood of Hemophilia A sub-type and confirmed that James does not suffer from Von Willebrand Disease.

Further testing indicated virtually absent Factor IX activity level, although no Factor IX Inhibitor was detected. Therefore, James appears to be afflicted with the Hemophilia B sub-type which is also known as Factor IX deficiency disease.

Raven exhibits similar lab test results – although more moderate in scale.

Both James and Raven’s genetic testing confirms the presence of a known pathogenic variant (F9:c.223C>T,p.R75X) in the Factor IX gene which is encoded on the X chromosome. As James has only one copy of the Factor IX gene (since he is male with only one X chromosome), this has dominant consequences in phenotype, while his mother is designated as a “Carrier” since she has been found to have one normal copy of the Factor IX gene and one variant copy. X chromosome mosaicism (due to somewhat random X chromosome inactivation in females) may explain the lesser bleeding issues that Raven exhibits, although there is some disease penetrance as evidenced by heavy menstrual flows and the requirement of a transfusion after childbirth.

Thus, we can diagnose James with Hemophilia B, also known as Factor IX deficiency disease, and Raven as a carrier of the Hemophilia B variant – which may impart some of the Hemophilia phenotype.

Many helpful literature resources and sources of genetic tests are available for Hemophilia B and can be found linked from the MedGen page: http://www.ncbi.nlm.nih.gov/medgen/945.

These include: GeneReviews and OMIM articles and Clinical Trials; relevant PubMed records identified as - Clinical Studies (separated by sections of Etiology, Diagnosis, Therapy, Prognosis and Clinical prediction guides) and Systematic reviews; and Patient Education Materials from Medline Plus, Genetics Home Reference, and NHLBI Health Topics.
The Factor IX gene is a critical participant in the intrinsic blood coagulation cascade pathway.

**GENE SUMMARY DESCRIPTION:** This gene encodes vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen. This factor is converted to an active form by factor Xla, which excises the activation peptide and thus generates a heavy chain and a light chain held together by one or more disulfide bonds. The role of this activated factor IX in the blood coagulation cascade is to activate factor X to its active form through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene, including point mutations, insertions and deletions, cause factor IX deficiency, which is a recessive X-linked disorder, also called hemophilia B or Christmas disease. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar proteolytic processing. [provided by RefSeq, Sep 2015]

![Diagram of the intrinsic and extrinsic pathways of blood coagulation]

This gene is expressed almost exclusively in the liver and then gets secreted into the bloodstream where it fulfills its physiological purpose.

The 461 amino acid-long gene product, Factor IX (also known as F9) has several annotated regions and established functional domains. For a detailed explanation of these regions, see the next page.

**James' Factor IX genetic variant (F9:c.223C>T,p.R75X)** occurs at position 75 in the protein sequence and converts an arginine (R) in the normal protein into a stop or termination codon (X) in the mRNA transcript. This early termination stops the production of the protein approximately 16% of the way into the protein.

When the protein synthesis stops at codon 75 to form a peptide containing only the first 74 amino acids, residues which normally form the critical functional domains in this protein are never added. This explains why the lab was unable to detect much Factor IX activity, and why James' clotting cascade isn't working well.

**Raven has one normal Factor IX gene and one that produces truncated Factor IX,** like James. Depending upon which X chromosome is active in her liver cells, she can produce some full-length and fully functional Factor IX protein — but not as much as she would if she had two non-variant copies.

For those interested in the story of Alexei Romanov & the "European Royal Disease"... you may want to take a look at these two articles which describe how they found the exact mutation founded in Queen Victoria and passed down to and through many members of European royalty, including to the little Tsarevich.

*Genotype analysis identifies the cause of the "royal disease"* - Science. 2009 Nov 6;326(5954):817.

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**ANNOTATED REGIONS:**

![Signal Peptide AA Features](http://1.usa.gov/1VQa3oT)

**FUNCTIONAL DOMAINS:**

![GLA, EGF_CA, FXa_inhibitory, Tryp_SPC superfamily](http://1.usa.gov/1ZGIHI5)

In the full-length pre-pro-protein, there is a signal peptide in the first 28 amino acids that directs the cell to export this protein out of the originating liver cell. As this is done, it is cleaved off to create a pro-protein form. The GLA (γ-carboxyglutamate) domain is the site of attachment of several types of post-translational modification which enable it to migrate to cell membranes. The EGF_CA domain binds to calcium ions near clots initiating a change of structure in the protein, which then exposes the GLA domain and the FXa_inhibitory Domain which allows for this protein to bind to a clot, but to bind to and not prematurely activate the next clotting factor in the chain. The cleavage site is the location for Factor XI to cut apart the first and second halves of the protein releasing the Trypsin-like Serine Protease Domain to now activate Factor X.

**GENE REVIEW'S NORMAL GENE PRODUCT DESCRIPTION:**

The factor IX gene product (reference sequence NP_000124.1) includes several distinct domains [Kanagasabai et al 2013, Rallapalli et al 2013]. The first and second domains are a signal peptide and a propeptide (respectively) that are cleaved to yield the mature protein, which is secreted as a single-chain peptide with 415 amino acid residues. Post-translational modifications include glycosylation, sulfation, phosphorylation, β-hydroxylation, and γ-carboxylation. A γ-carboxylase binds to the propeptide before cleavage and, in a vitamin K-dependent step, converts the first 12 glutamic acid residues (near the amino-terminus) to γ-carboxyglutamatic residues (GLA domain). This GLA domain then binds calcium ions and adopts a conformation capable of binding to a phospholipid surface where the clotting cascade occurs. Adjacent to the GLA domain are two domains homologous with epidermal growth factor. The next domains are a connecting sequence that includes the activation peptide, and finally the catalytic domain. The latter is typical of serine proteases. Crystal structures are consistent with other data that show the catalytic domain elevated above a lipid surface. Factor IX is homologous with clotting factors VII and X and protein C.

Factor IX is synthesized in hepatocytes and circulates as a zymogen at 90 nmol/L (5 µg/mL). During coagulation in vivo, it is activated by factor VIIa tissue factor in a reaction in which the activation peptide is cleaved. Activated factor IX is the intrinsic factor X activator, requiring its cofactor, activated factor VIII, a lipid surface, and calcium. Sites of interaction of the active enzyme and cofactor are being identified [Kanagasabai et al 2013]. Factor X activation is a critical early step that can regulate the overall rate of thrombin generation in coagulation.