	 Marco	 Alexei	 James	 Bo
Reported Phenotype	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: • Maternal uncle died at the age of 6 years old from a "brain bleed" after a fall. • Mother required a blood transfusion after natural childbirth	laceration on left index finger with prolonged bleeding 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)*
Reported 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**
Variant Information: • Asserted interpretation listed in **ClinVar**	not in ClinVar! (assumed a VUS)	pathogenic	pathogenic	pathogenic
• HGVS names from **ClinVar**	**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**?	Searching directly in dbSNP found no records	rs398122990 Yes! And it is really, really rare.	rs387906432 Yes! And it is really, really rare.	rs137852234 Yes! And it is pretty darn rate.
Gene Information in NCBI Gene: • Gene Symbol & Name	F8 & Coagulation factor VIII	F9 & Coagulation factor IX	F8& Coagulation factor VIII	F9 & Coagulation factor IX

	Marco	Alexei	James	Bo
Gene Information in NCBI Gene (con't): • Gene Summary	F**8** …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]	**F9** …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]	**F8**…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]	**F9** …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]
• Tissue Expression information	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
• Gene Ontology information	Extracellular Blood coagulation Protein binding	Extracellular Blood coagulation Ca+2-binding & endopeptidase	Extracellular Blood coagulation Protein binding	Extracellular Blood coagulation Ca+2-binding & endopeptidase
Ultimate Impacted Biomolecule based on: • **GDV** to view the chromosome and gene region	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.
• **RefSeqGene Graphics** view of gene region and transcript(s)	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.
• **RefSeq Protein Graphics** view of protein and domains	n/a	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.
• **CDD or iCn3D** to view a structure	n/a	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 identified as one of 3 residues annotated as critical for binding to Ca+2. The change from acidic Aspartate residue to neutral Glycine likely prevents its participation.
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 aggregating 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.