Complexities and resolution of gene variant interpretation in two hemophilia cases

ML Alabek[1], SM Ghate[2], R Udani[3], KD Friedman[3], MW Anderson[3], LM Malec[1], LC Palmer[2], MV Ragni[1,4], and SN Dugan[3]

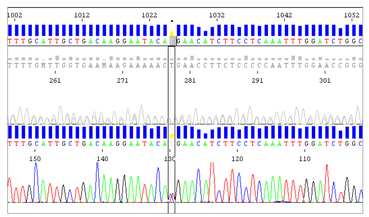
Hemophilia Center of Western Pennsylvania, Pittsburgh, PA[1], Hemophilia Outreach Center, Green Bay, WI[2], BloodCenter of Wisconsin, Milwaukee, WI[3], and University of Pittsburgh, Pittsburgh, PA[4]

Introduction and Objectives:

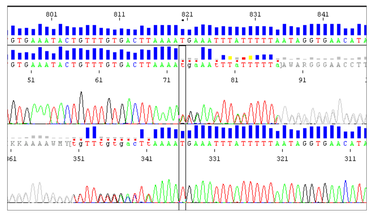
Despite being well-conserved genes, not all variants in *F8* and *F9* are pathogenic. Even when testing is targeted for a familial pathogenic variant, additional variants may be found. The interpretation and classification of any genetic variant is critical to appropriately direct management and inform genetic counseling for individuals and their relatives. We describe two hemophilia cases for which thorough expert analysis resulted in additional recommendations to further inform result interpretation, ultimately allowing for more clinically actionable genetic test results.

Methods and Materials:

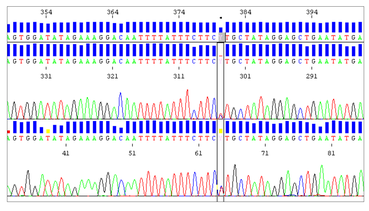
Case 1 is a 21-year-old female of Amish ancestry with family history of moderate hemophilia B. *A priori* risk to be a carrier was 50%; baseline FIX level was 111%; bleeding symptoms were denied. Case 2 is a 21-year old female of Mexican ancestry with menorrhagia and a family history of mild-moderate hemophilia A. *A priori* risk to be a carrier was 50%; baseline FVIII levels ranged from 46% to 138% from different laboratories. Targeted genetic testing by sequencing did not identify the familial pathogenic variant in case 1 (FPV1)[1] or case 2 (FPV2)[2,3], but did identify a separate intronic variant in case 1 (IV1) and case 2 (IV2). IV1[4,5] and IV2[6,7,8] were reported in gene-specific databases and the literature, but pathogenicity was unclear. In attempt to classify the unanticipated variants, various additional sources of evidence were assessed through collaboration by the laboratory and ordering clinicians.



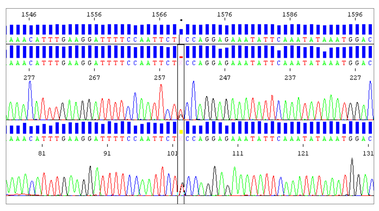
IV1 detected in patient case 1:
F9 c.839-20dupA, heterozygous, intron 7



FPV1 detected in carrier sister of case 1:
F9 c.1025C>T, heterozygous, exon 8

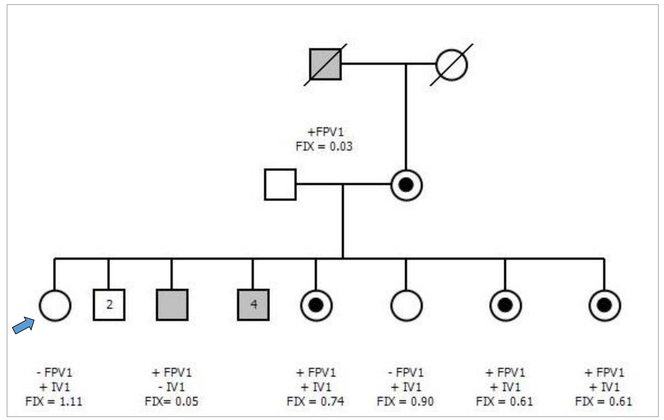


IV2 detected in patient case 2:
F8 c.389-9C>T, heterozygous, intron 3



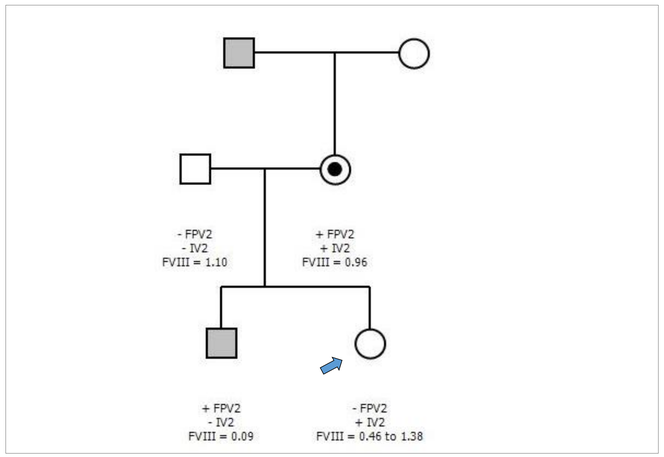
FPV2 detected in carrier mother of case 2:
F8 c.1569G>T, heterozygous, exon 11

PEDIGREE 1 AND RESULTS



= hemophilia = carrier of hemophilia
FPV1 = familial pathogenic variant *F9* c.1025C>T (p.T342M), exon 8
IV1 = intronic variant *F9* c.839-20dupA, intron 7
FIX = factor IX level (units = IU/ml)

PEDIGREE 2 AND RESULTS



= hemophilia = carrier of hemophilia
FPV2 = familial pathogenic variant *F8* c.1569G>T (p.L523L), exon 11
IV2 = intronic variant *F8* c.389-9C>T, intron 3
FVIII = factor FVIII level (units = IU/ml)

Results:

In case 1, variant segregation analysis from relatives' concurrent testing suggested that IV1 was paternally inherited and in *trans* with FPV1. Interpretation of available evidence led to classifying IV1 as a variant of uncertain significance. The father reported no personal or maternal family history of bleeding symptoms. FIX activity level and targeted testing for IV1 in the father were recommended to further inform classification of this variant; however, the family was lost to follow-up prior to completion of this testing. In case 2, reflexive maternal and paternal testing allowed for variant segregation analysis and correlation with FVIII activity level (FVIII:C). The mother was determined to carry both FPV2 and IV2 *in trans*; her factor VIII:C level was 96%. Interpretation of this evidence led to classifying IV2 as likely benign.

	Case 1	Case 2
Family diagnosis	hemophilia B, moderate	hemophilia A, mild-moderate
Familial pathogenic variant (FPV)	*F9* c.1025C>T (p.T342M), exon 8 [1]	*F8* c.1569G>T (p.L523L), exon 11 [2,3]
Testing performed on patient	targeted sequencing of *F9* exon 8	full *F8* coding sequencing; followup targeted sequencing of *F8* exons 4 and 11
Intronic variant (IV)	*F9* c.839-20dupA, intron 7 [4,5]	*F8* c.389-9C>T, intron 3 [6,7,8]
Supporting evidence used for reclassification	• Family segregation studies • Genotype/phenotype correlation with baseline factor levels: not available • *in silico* models	• Family segregation studies • Genotype/phenotype correlation with baseline factor levels • *in silico* models
Final classification of intronic variant	uncertain significance	likely benign

Table 1: Summary of detected variants in *F8* and *F9*

Conclusions:

Variant classification influences clinical utility for affected individuals and relatives. Rigorous scrutiny and close collaboration between the performing laboratory and ordering clinicians can further inform classification to impact result interpretation and appropriate testing for at-risk family members. In other cases, insufficient evidence to confirm pathogenicity may remains despite best efforts, limiting clinical utility of the test result until further supporting evidence can be obtained.

References/Bibliography:

1.Chen SH˙Zhang M, Lovrien EW, Scott CR, Thompson AR. CG dinucleotide transitions in the factor IX gene account for about half of the point mutations in hemophilia B patients: a Seattle series. Hum Genet. 87(2):177-82, 1991 Jun. **2.**Economou EP, Kazazian HH Jr, Antonarakis SE. Detection of mutations in the factor VIII gene using single-stranded conformational polymorphism (SSCP). *Genomics* 1992; 13: 909–11. **3.**Tavassoli, K., Eigel, A., Wilke, K., Pollmann, H. and Horst, J. (1998), Molecular diagnostics of 15 hemophilia A patients: Characterization of eight novel mutations in the factor VIII gene, two of which result in exon skipping. Hum. Mutat., 12: 301–303. **4.**Ghanem N, Costes B, Martin J et al. Twenty-four novel hemophilia B mutations revealed by rapid scanning of the whole factor IX gene in a French population sample. Eur J Hum Genet 1993; 1: 144-55. **5.** CDC Hemophilia A Mutation Project (CHAMP) and and CDC Hemophilia B Mutation Project (CHBMP): http://www.cdc.gov/ncbddd/hemophilia/champs.html. **6.**HGMD® Professional 2016.2: https://portal.biobase-international.com/hgmd/pro **7.** EAHAD Factor VIII Variant Database: http://www.factorviii-db.org/. **8.**Timur, A. A. , Gürgey, A. , Aktug˘lu, G. , Kavakli, K. , Canatan, D. , Olek, K. and Çag˘layan, S. H. (2001), Molecular pathology of haemophilia A in Turkish patients: identification of 36 independent mutations. Haemophilia, 7: 475–481.