Title

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Journal

Research and Practice in Thrombosis and Haemostasis, 4(5)

ISSN

2475-0379

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Publication Date

2020-07-01

DOI

10.1002/rth2.12345

Peer reviewed

Received: 27 January 2020 | Revised: 4 March 2020 | Accepted: 19 March 2020

DOI: 10.1002/rth2.12345

CASE REPORT

rpth research & practice in thrombosis & haemostasis

The odds and implications of coinheritance of hemophilia A and B

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Handling Editor: Dr Cihan Ay

Abstract

We report 2 patients with coinheritance of the X-linked bleeding disorders hemophilia A and hemophilia B. We describe the family pedigrees, clinical features, and genotyping. The case report addresses the key clinical questions of how to manage patients with both hemophilia A and B and how to counsel families regarding recurrence risk. The patients with coinherited hemophilia A and B require a combination of factor VIII and factor IX replacement to achieve hemostasis. We calculated the estimated genomic meiotic recombination frequency between *F8* and *F9* to be 38%. The findings in these cases are consistent with this calculation. These findings provide critical information for management of families with coinherited hemophilia A and B.

KEYWORDS

factor IX, factor VIII, genetic counseling, hemophilia, pedigree, recombination genetic

Essentials

- Hemophilia A and B are rare X-linked inherited bleeding disorders that may be coinherited.
- We describe 2 cases of coinherited hemophilia A and B.
- Management requires novel treatment approaches.
- We estimate the recombination rate between the *F8* and *F9* loci to be ~38%.

1 | BACKGROUND

Congenital hemophilia A (HA) and B (HB) are rare X-linked recessive disorders, occurring in approximately 1 in 5000 and 1 in 30 000 live male births, respectively.[1] Combined inheritance of HA and HB is even more rare, but has been reported due to inheritance of 1 variant from each parent[2] or due to 2 variants from a single parent.[3-6] We report 2 families with pathogenic genetic variants for HA and HB and evaluate how the variant *F8* and *F9* alleles were inherited within the families. In addition, we review implications for diagnosis, genetic counseling, and management.

2 | CASE DESCRIPTIONS

2.1 | Family 1

An 11-month-old male presented with bleeding from a torn frenulum without other bleeding history. The mother reported a history of heavy menstrual bleeding, postpartum hemorrhage, and bleeding with dental work. There was no other family history of bleeding, including the proband's fraternal twin brother. Laboratory results showed a normal PT of 10.6 seconds, an activated partial thromboplastin time (APTT) of 124.2 seconds (normal, 24.0-34.0), which corrected on mixing. factor VIII (FVIII) activity was <1% and factor IX (FIX) activity was 23%. Results were normal for factors XI (FXI) and von Willebrand factor (VWF) antigen and activity. This led to a diagnosis of severe HA and mild HB. The patient was initially treated with recombinant FVIII (rFVIII) replacement for bleeding. At age 21 months, after ~3 exposure days (EDs), a low-titer, low-responding FVIII inhibitor was identified on surveillance labs. The inhibitor was managed with immune tolerance induction (ITI) with rFVIII. Once tolerance was achieved, he was switched to prophylaxis without inhibitor recurrence. He also received on-demand recombinant FIX (rFIX) for bleeding unresponsive to rFVIII replacement and for major bleeding and surgery. He has no history of FIX inhibitor.

The mother has mild FVIII deficiency (42%) and normal FIX activity (62%) (Table 1). The laboratory evaluation of the proband's fraternal twin showed an APTT of 34.9 seconds; FIX activity of 27%; and normal FVIII, FXI, and VWF antigen and activity, consistent with

TABLE 1 Coagulation testing, genotypes, and clinical phenotypes of family 1 and family 2

	APTT,[a] seconds	FVIII activity,[a] %	*F8* variant	FIX Activity,[a] %	*F9* Variant	Clinical phenotype
Family 1						
I.1	…	42	*F8* deletion of exon 26 and 3′-UTR	69	*F9* c.[277 + 102G > C;*1368A > C]	Menorrhagia, postpartum hemorrhage, excessive bleeding from dental work (age 43 y)
II.1	34.3	94	ND	70	ND	No bleeding (age 24 y)
II.2	32.6	87	ND	55	ND	No bleeding (age 23 y)
II.3	31.6	108	ND	76	ND	No bleeding (age 22 y)
II.4	124.2	<1	*F8* deletion of exon 26 and 3′ UTR	23	*F9* c.[277 + 102G > C;*1368A > C]	Hemarthrosis, soft tissue bleeds, and excessive bleeding from dental work in the absence of FIX replacement Treatment: prophylactic and on-demand rFVIII; on-demand rFIX (age 17 y)
II.5	34.9	99	None detected	27	*F9* c.[277 + 102G > C;*1368A > C]	Soft tissue and mucosal bleeding secondary to trauma Treatment: on-demand rFIX (age 17)
II.6	30.2	165	ND	70	ND	No bleeding (age 19 y)
II.7	…	47	ND	52	ND	No bleeding (age 13 y)
II.8	117.2	<1	*F8* deletion of exon 26 and 3′-UTR	47 at birth; 80 on repeat at 1 y	None	Ecchymosis, hemarthrosis Treatment: prophylactic and on-demand rFVIII (age 7 y)
II.9	…	…	ND	ND	ND	No bleeding (age 3 y)
Family 2						
I.1	-	reportedly normal during pregnancy	*F8* c.6089G > A	48	*F9* partial deletion of exon 8	Easy bruising and heavy menstrual bleeding (age 27 y)
II.1	>100	21	*F8* c.6089G > A	<1	*F9* partial deletion of exon 8	Bruising, mucosal bleeding, hemarthrosis; FIX inhibitor Treatment: ITI with rFIXFc plus prophylactic rFVIIa and rFVIIIFc (age 2 y)
II.2	49 (within normal newborn range)	72	ND	29 (within normal newborn range)	ND	No bleeding (age 6 m)

Note: APTT, activated partial thromboplastin time; FIX, factor IX; FVIII, factor VIII; ITI, immune tolerance induction; ND, testing not completed or not available; rFIX, recombinant factor IX; rFVIII, recombinant factor VIII; UTR, untranslated region.

[a]Normal range: APTT range, 25.0–34.0 s; FVIII activity range, 60%–150%; FIX activity range, 60%–150%.

a diagnosis of mild HB. A younger half-brother was diagnosed with only severe HA as a neonate.

Genotyping of *F8* and *F9* was done with My Life, Our Future (Table 1 and Figure 1).[7,8] The *F8* variant identified, c.6901-?_7056+?del, resulting in deletion of exon 26 and the 3′-untranslated region, has previously been reported in multiple families with severe HA, meeting criteria to be classified as a pathogenic variant.[9] Two *F9* variants were identified and cosegregated in multiple family members, c.[277 + 102G > C, *1368A > C]. Both variants are absent in population databases.[10-12] These were considered variants of unknown significance in the absence of additional American College of Medical Genetics criteria supporting pathogenicity.[13]

2.2 | Family 2

A 2-month-old male was evaluated for easy bruising and scalp hematoma without other bleeding history. The mother and maternal grandmother reported a history of easy bruising and heavy menstrual bleeding. There was no other family history of bleeding, including in many maternal male relatives. Laboratory results showed a PT of 12.7 seconds and an APTT of >100 seconds, which corrected on mixing. FVIII activity was 35% (21% on repeat plasma-based assay and 17% on chromogenic assay) and FIX activity was <1%. Results were normal for FXI and VWF antigen and activity. This led to a diagnosis of severe HB and mild HA (Table 1). The patient was initially treated with rFIX replacement for bleeding. At 9 months of age, after 3 EDs, a 1.3-BU FIX inhibitor was identified on surveillance labs; repeat was 0.7 BU. On ED 5, he developed anaphylaxis and lack of clinical response in the setting of traumatic mouth bleed; inhibitor was 4.7 BU. He started ITI with daily rFIXFc after desensitization. After 2 months, his inhibitor was negative at 12 months, and after 11 months his pharmacokinetics were sufficient for every-other-day dosing. Initially, daily recombinant factor VIIa (rFVIIa) and twice weekly rFVIIIFc were given prophylactically; rFVIIa was discontinued once inhibitor was negative and he had detectable trough FIX activity.

The mother had only FVIII and IX activity testing during pregnancy; results were reportedly normal. The second son had FVIII and IX activities that were normal for his age.

Genotyping of *F8* and *F9* identified a missense pathogenic variant in *F8* (c.6089G > A; p.Ser2030Asn), which was previously reported in multiple families with hemophilia A,[9,14,15] and a pathogenic partial deletion of exon 8 in *F9*, which was not previously described (Figure 1). The mother's genetic testing confirmed she carries the *F8* variant and the *F9* deletion. Both are presumed to be de novo since the maternal grandmother tested negative for the 2 variants identified in the family, and the maternal grandfather has a negative bleeding history.

The *F9* deletion appears to be a de novo somatic variant that likely occurred in early embryogenesis given the presence of the deletion at a lower level in leukocyte DNA.

3 | RESULTS AND DISCUSSION

The human X chromosome shows an average rate of meiotic recombination (exchange of genetic information between chromosomes

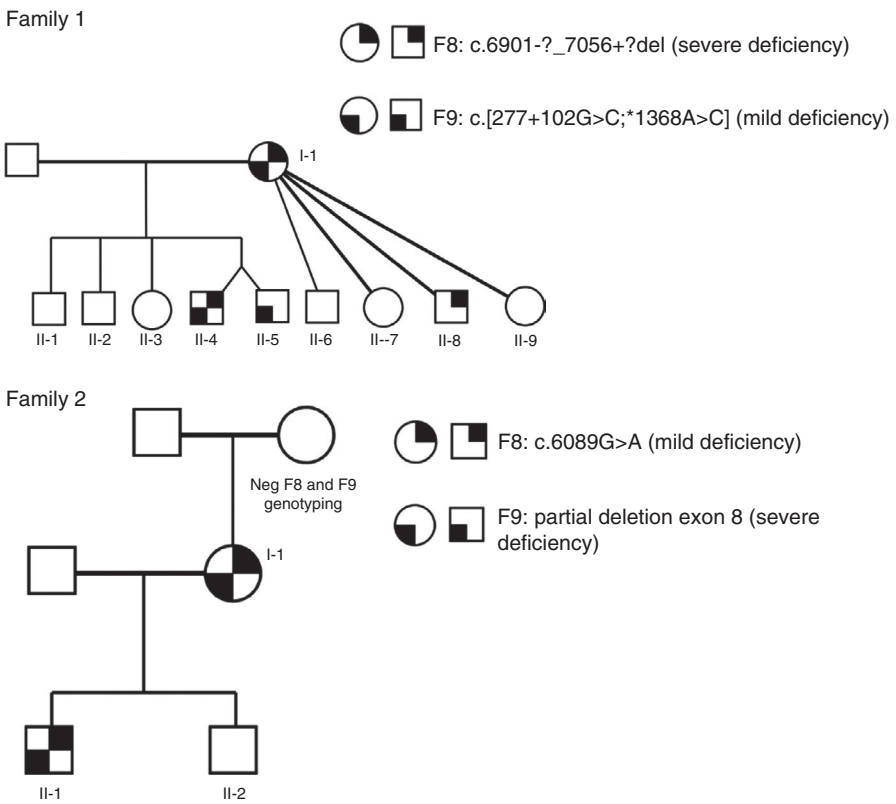

FIGURE 1 Family pedigrees. Family pedigrees are demonstrated and include available genotyping results. Confirmed affected family members are indicated with shading for hemophilia A, hemophilia B, or both. For family 1, males II-1, II-2, and II-6 are presumed to have normal genotype based on factor levels. Genotypes are unknown for females II-3, II-7, and II-9. For family 2, male II-2 is presumed to have normal genotype based on factor levels

during meiosis) of 1.19% per megabase (1.19 cM/Mb). The *F8* locus (Xq28;154.8-155.0 Mb [human hg38 genome assembly]) and *F9* locus (Xq27.1-27.2; 139.53-139.56 Mb) loci are located at a physical distance of ~15.5 Mb apart on the q arm of the X chromosome. Using the average recombination rates of the X chromosome, the predicted recombination rate would be 15%.[16] However, the interval close to the X-chromosome telomere, inclusive of *F8* and *F9*, shows a markedly higher recombination rate of ~2.46 cM/Mb.[16,17] Based on this information, we estimate the recombination rate between the *F8* and *F9* loci to be ~38% (15.5 Mb × 2.46 cM/Mb) (Figure 2).

We describe 2 cases of coinherited HA and HB. In family 1, there were 6 males and 3 females born to the mother. The females were not genotyped and were otherwise uninformative. The mother is heterozygous for variants in both *F8* and *F9* genes, and these could either lie in *cis* (on the same chromosome) or in *trans* (on opposite chromosomes). Four sons inherited either both or neither *F8* and *F9* alleles, while two sons inherited one *F8* or *F9* hemophilia-associated allele. If the *F8* and *F9* variant alleles lie in *cis* in the mother, with recombination giving rise to the 2 offspring separating 1 *F8* or *F9* allele each, the observed meiotic recombination rate is 33%. If the mother's variant alleles lie in *trans*, then 4 meiotic recombination events would be required for males to have alleles align in *cis*, giving a recombination rate of 67%. Given the calculated genomic meiotic recombination rate of 38% between these genes, we suspect that the mother's variant alleles are in *cis*, but either way recombination between the *F8* and *F9* genes in this family was common.

In any female family member at-risk for having disease-causing variants in both the *F8* and *F9* genes, prenatal counseling must include the possibilities of passing on none, both, or a single variant hemophilia-causative allele, depending on whether recombination occurs between the genes.[7] For potentially affected females, we recommend testing factor activities by age 2 years. Some female hemophilia genetic carriers bleed excessively even with normal factor levels,[18] making it reasonable to offer genetic testing before adolescence if there is bleeding or need for invasive procedure even if levels are normal.

In family 1, the mother and her twin boys had the two *F9* variants. It is possible that only 1 of these variants is pathogenic. With the available information, it is impossible to assess the pathogenicity of these 2 variants independently because neither variant has been reported individually.[10-12]

In family 2, the mother carries 2 variants that were not identified in her mother. Her father was not tested but is clinically unaffected by report. The *F8* missense variant appears to be a de novo germline variant that was likely present in the sperm or the egg at the time of her conception. The *F9* deletion appears to be a de novo somatic variant that likely occurred in early embryogenesis given the presence of the deletion at a lower level in leukocyte DNA. Given the likely somatic mosaicism of the *F9* variant, the risk for recurrence is further complicated, as it is unknown what percentage of oocytes carries the variant. The mother's second pregnancy was managed in close collaboration with obstetrics and hematology since the male fetus was potentially affected by severe HB and/or mild HA.

Combined factor deficiencies are likely underestimated, and comprehensive coagulation testing is recommended for all patients and siblings for accurate diagnosis and treatment.[3] Patients with HA and HB need FVIII and FIX replacement. We are considering switching from rFVIII prophylaxis to emicizumab for the proband in family 1 with severe HA and mild HB, though it is unclear if mild FIX deficiency would blunt the hemostatic efficacy of this bispecific antibody treatment. There are emerging novel treatments for patients with HA or HB that generate thrombin without factor replacement.[19,20] Patients with combined HA and HB could be excellent candidates for these novel treatments.

In summary, recurrence risk counseling is complex when there are 2 X-linked disorders within a family, particularly in the absence of genotypes for all potentially affected individuals. Due to the possibility of meiotic recombination, one cannot assume that 2 disorders are present in *cis* in

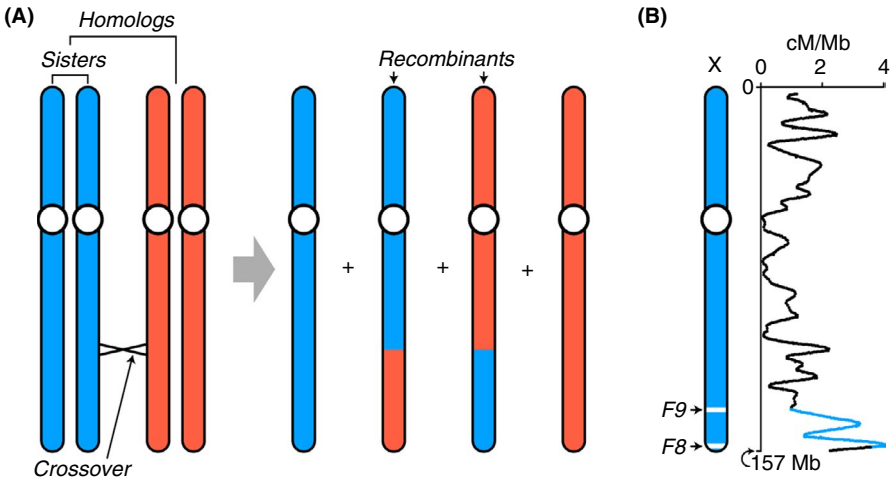

FIGURE 2 Meiosis schematic for hemophilia. (A) Schematic of 1 pair of homologs (each replicated into a pair of sister chromatids) as they align and recombine in meiotic prophase. Each homolog pair receives 1-2 meiotic crossovers (marked), which result in recombinants. White circles indicate the location of the centromere, which mediates spindle attachment during meiosis. (B) Schematic of the human X chromosome, with the *F8* and *F9* loci marked. At right is the measured recombination rate in cM/Mb (adapted from Kong et al[16]), with the interval between *F8* and *F9* highlighted in blue. This interval shows a higher average recombination rate (2.46 cM/Mb) than the X chromosome as a whole (1.19 cM/Mb)

a female relative when a male first-degree relative is affected with both conditions. We calculated a high genomic meiotic recombination frequency of 38% between the *F8* and *F9* genes, and further discuss the inheritance and management implications of this phenomenon in 2 families. We expect this work will be useful to inform the estimations of recurrence risk for hemophilia A and B in individual families.

ACKNOWLEDGMENTS

The authors thank the families for allowing us to share the clinical and genetic histories. The authors thank the Rady Children's Hospital San Diego HTC team for providing comprehensive care to the hemophilia community.

RELATIONSHIP DISCLOSURE

CDT reports personal fees from Genentech, grants and personal fees from Sanofi Genzyme, personal fees from Spark Therapeutics, grants and personal fees from NovoNordisk, grants from Octapharma, grants from CSL Behring, and grants from Shire, outside the submitted work. The other authors have no conflicts of interest to report.

AUTHOR CONTRIBUTIONS

CK, DM-F, and CDT contributed to concept and design, data collection, analysis and interpretation of the data, critical writing and revising of the manuscript, and final approval. SER, KDC, and JMJ provided expertise to the data analysis and interpretation, revising of the manuscript, and final approval. SNF performed experiments and data analysis and provided final approval. JL contributed to data collection, revising of the manuscript, and final approval.

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REFERENCES

1. Swystun LL, James PD. Genetic diagnosis in hemophilia and von Willebrand disease. Blood Rev. 2017;31(1):47–56.
2. Shetty S, Ghosh K, Parekh S, Mohanty D. Combined factor VIII and IX deficiency in a family. Clin Lab Haematol. 2001;23(3):201–4.
3. Prabhudesai A, Shanbhag S, Mirgal D, Kawankar N, Shetty S. A de novo factor VIII mutation in a haemophilia B family leading to combined deficiency of factor VIII and IX. Hemophilia. 2017;23(5):e477–79.
4. Siddiq S, Morse C, Goodeve A, Panayi M, Tait RC, Mumford A. F8 and F9 mutations fail to co-segregate in a family with co-incident haemophilia A and B. Haemophilia. 2011;17(1):e230–34.
5. Ivaskevicius V, Pezeshkpoor B, Biswas A, Goldmann G, Horneff S, Gimbutyte M, et al. Combined coagulation factor VIII and factor IX deficiency (CDF8F9) in a patient from Lithuania. Hamostaseologie. 2016;36(Suppl. 2):S29–33.
6. Lu Y, Xie B, Ding Q, Dai J, Xi X, Wang M, et al. Occurence of haemophilia A and B in a Chinese family with mosaicism of the F9 gene mutation in the HB index's maternal grandfather. Thromb Haemost. 2010;103(5):1106–8.
7. Johnsen JM, Fletcher SN, Huston H, Roberge S, Martin BK, Kircher M, et al. Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the My Life, Our Future initiative. Blood Adv. 2017;1(13):824–34.
8. Konkle BA, Johnsen JM, Wheeler M, Watson C, Skinner M, Pierce GF, et al. Genotypes, phenotypes and whole genome sequence: approaches from the My Life Our Future haemophilia project. Hemophilia. 2018;24(Suppl 6):87–94.
9. European Association for Haemophilia and Allied Disorders. Factor VIII gene (F8) variant database. http://f8-db.eahad.org/. Accessed 30 July 2018.
10. Centers for Disease Control and Prevention. CDC Hemophilia Mutation Database Project (CHBMP). https://www.cdc.gov/ncbddd/hemophilia/champs.html. Accessed 04 October 2019.
11. Rallapalli PM, Kemball-Cook G, Tuddenham EG, Gomez K, Perkins SJ. An interactive mutation database for human coagulation factor IX provides novel insights into the phenotypes and genetics of hemophilia B. JTH. 2013;11(7):1329–40.
12. Karczewski KJ, Francioli LC, Tiao G, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. bioRxiv. 2019. https://doi.org/10.1101/531210
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
14. Lannoy N, Lambert C, Vikkula M, Hermans C. Overrepresentation of missense mutations in mild hemophilia A patients from Belgium: founder effect or independent occurrence? Thromb Res. 2015;135(6):1057–63.
15. Liu M, Murphy ME, Thompson AR. A domain mutations in 65 haemophilia A families and molecular modelling of dysfunctional factor VIII proteins. Br J Haematol. 1998;103(4):1051–60.
16. Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, et al. A high-resolution recombination map of the human genome. Nat Genet. 2002;31(3):241–47.
17. Davies KE, Mattei MG, Mattei JF, Veenema H, McGlade S, Harper K, et al. Linkage studies of X-linked mental retardation: high frequency of recombination in the telomeric region of the human X chromosome (fragile site/linkage/recombination/X chromosome). Hum Genet. 1985;70(3):249–55.
18. James PD, Mahlangu J, Bidlingmaier C, Mingot-Castellano ME, Chitlur M, Fogarty PF, et al. Evaluation of the utility of the ISTH-BAT in haemophilia carriers: a multinational study. Hemophilia. 2016;22(6):912–18.
19. Callaghan MU, Sidonio R, Pipe SW. Novel therapeutics for hemophilia and other bleeding disorders. Blood. 2018;132(1):23–30.
20. Sehgal A, Barros S, Ivanciu L, Cooley B, Qin J, Racie T, et al. An RNAi therapeutic targeting antithrombin to rebalance the coagulation system and promote hemostasis in hemophilia. Nat Med. 2015;21(5):492–7.

How to cite this article: Karch C, Masser-Frye D, Limjoco J, et al. The odds and implications of coinheritance of hemophilia A and B. *Res Pract Thromb Haemost*. 2020;4:931–935. https://doi.org/10.1002/rth2.12345