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Discordant hemophilia A in male siblings due to a de novo mutation on a familial missense mutant allele

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Hemophilia A is an X-linked disorder of hemostasis caused by mutations of the factor VIII gene. Discordance of phenotype in male siblings is relatively unusual when both carry a familial mutation.

The propositus came to medical attention as a newborn infant after prolonged bleeding from a heel capillary puncture. Subsequent evaluation revealed normal platelet number and function, severely prolonged partial thromboplastin time (PTT), normal von Willebrand factor (vWF) antigen and activity, and reduced factor VIII activity of 1.7 % (Table 1, Brother 1). His clinical history has been notable for significant easy bruising and arterial pseudoaneurysm of the wrist, dating from the phlebotomy for newborn bleeding assessments. His clinical syndrome is consistent with a diagnosis of severe hemophilia A.

The evaluation of brother 1 led to testing of his 4 year old, full sibling which revealed mildly prolonged PTT and factor VIII activity of 26 %, consistent with his asymptomatic clinical history (Table 1, Brother 2). There was no other known family history of bleeding disorders. Indeed, testing of both parents revealed normal factor VIII activities.

We reasoned that the two children had distinct phenotypes, and could have distinct genetic abnormalities. Evaluation of both brothers revealed no functional evidence of von Willebrand disease. Moreover, brother 2 had no evidence of genetic mutations associated with type IIN disease, which would have explained his reduced factor VIII activity, as caused by enhanced proteolysis of factor VIII.

In the absence of demonstrable explanation of reduced factor VIII activity of brother 2 due to causes other than hemophilia A, we postulated that he must have a familial mutation of his factor VIII gene associated with a mild phenotype, which must be shared by brother 1 and his mother, with brother 1 having an additional mutation, conferring a severe phenotype.

DNA sequencing of the factor VIII gene of brother 2 revealed a hemizygous mutation c. 1094A>G in exon 8, leading to a missense mutation p.Tyr346Cys, previously reported to be

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associated with a mild phenotype. An identical mutation was detected in his mother. Factor VIII gene sequencing of brother 1 revealed not only the expected 1094A>G, but also an additional mutation c.3870delA in exon 14, expected to lead to a nonsense frameshift with a premature stop codon, consistent with his severely reduced factor VIII activity and phenotype. Thus, the two brothers and their mother, who is an asymptomatic carrier, share a familial missense mutation of the factor VIII gene associated with a mild phenotype, with brother 1 having acquired an additional, severe nonsense mutation, arising de novo in the maternally inherited mutant allele.

Among all patients with severe hemophilia A, mutations arising de novo most often involve inversions of intron 22, but these tend to arise during spermatogenesis and thus cannot originate in mothers, instead most often inherited from maternal grandfathers [1, 2]. Nonetheless, for life-threatening X-linked mutations that are expected to be at equilibrium in the population, like those causing hemophilia A, nearly 1/3 of mutations are expected to arise de novo [3]. Alleles causing hemophilia and arising de novo from a pre-existing hemophilia allele are expected to be very rare. Several patients with two mutations have been described for hemophilia B, caused by mutations of the factor IX gene, but it is not clear whether the second mutations are hemophilia alleles or neutral variants [4, 5]. There are no published reports of such mutations for hemophilia A, though several cases of new mutations arising de novo in the setting of familial hemophilia have been described in females as a result of different mutations of paternally and maternally inherited factor VIII genes [6–8], as well as non-random X chromosome inactivation [9]. Insofar as the discordant hemophilia A in the studied male sibling pair is the result of combined de novo and familial hemophilia alleles, its incidence in the general population is expected to be of a similar order as that of female hemophilia A usually caused by a combination of de novo mutation and inheritance of a familial hemophilia allele. This is a rare situation which has a high chance of being ascertained in a hemophilia treatment center, suggesting that: (i) in genetic counseling settings, the possibility of de novo mutations must be considered (even though they are rare); and (ii) in the case of discordant clinical presentations of hemophilia, testing only for a (known) familial mild allele may not be sufficient.

References

1. Rossiter JP, Young M, Kimberland ML, et al. Factor VIII gene inversions causing severe hemophilia A originate almost exclusively in male germ cells. Hum Mol Genet. 1994; 3:1035–9. [PubMed: 7981669]
2. Tizzano EF, Domenech M, Baiget M. Inversion of intron 22 in isolated cases of severe hemophilia A. Thromb Haemost. 1995; 73:6–9. [PubMed: 7740498]
3. Haldane JB. The rate of spontaneous mutation of a human gene. J Genet. 1935; 31:317–26.
4. Poort SR, Briet E, Bertina RM, Reitsma PH. Two mutations of the factor IX gene including a donor splice consensus deletion and a point mutation in a Dutch patient with severe hemophilia B. Thromb Haemost. 1990; 64:379–84. [PubMed: 2096489]
5. 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. 1991; 87:177–82. [PubMed: 2066105]
6. Cai XH, Wang XF, Dai J, et al. Female hemophilia A heterozygous for a de novo frameshift and a novel missense mutation of factor VIII. J Thromb Haemost. 2006; 4:1969–74. [PubMed: 16805874]

7. Vencesla A, Fuentes-Prior P, Baena M, Quintana M, Baiget M, Tizzano EF. Severe haemophilia A in a female resulting from an inherited gross deletion and a de novo codon deletion in the F8 gene. Haemophilia. 2008; 14:1094–8. [PubMed: 18665854]

8. Windsor S, Lyng A, Taylor SA, Ewenstein BM, Neufeld EJ, Lillicrap D. Severe haemophilia A in a female resulting from two de novo factor VIII mutations. Br J Haematol. 1995; 90:906–9. [PubMed: 7669670]

9. Bennett CM, Boye E, Neufeld EJ. Female monozygotic twins discordant for hemophilia A due to nonrandom X-chromosome inactivation. Am J Hematol. 2008; 83:778–80. [PubMed: 18645989]

Table 1

Laboratory evaluation

	Brother 1	**Brother 2**	**Mother**	**Father***
fVIII level (%)	1.7	26	93	127
PTT (sec)	101	37		
Inhibitor screen	Negative	Not done		
VWF gene sequencing for type IIN variants	Not done	Negative		
Blood type	O+	O+		
vWF Ag (%)	101	97		
vWF R:Co (%)	101	61		
vWF multimers	Normal	Normal		

*evaluated initially in consideration of type IIN vWD.