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LETTER TO THE EDITOR

A large deletion, spanning exons 1 to 25 of *F8* gene, and a high-titer factor VIII inhibitor, in severe hemophilia A

Dear Editor,

Hemophilia A (HA) is the most common severe congenital bleeding disorder; worldwide estimated incidence is one per 5000 male births.[1,2] According to the residual plasma level of factor (F) VIII level, patients with HA have variable clinical manifestations, ranging from asymptomatic condition to life-threatening diathesis. HA primarily is due to *F8* pathogenic variants, most commonly intron-1 and -22 inversions.[3] Patients without these inversions have pathogenic variants in other parts of the long *F8* gene. Although gross gene defects have been reported in the literature, entire *F8* gene deletion has not been reported yet.[1,2] In the present study, we described an Afghan family with severe HA and exon 1-to-25 deletion.

Initially, the study was started on a family member with a typical history of severe clinical presentations like recurrent hemarthrosis and hematoma. It was continued on other family members. Initially, physical examination was performed by a physician, and a standard questionnaire was completed by expert staff in order to determine age, sex, clinical presentations, and family history of bleeding. Routine tests, including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and platelet count, were performed in Afghanistan. Further studies like factor activity assays by one-stage PT-based and PTT-based assays were performed in Iran and Pakistan. Factor VIII (FVIII) inhibitor titer was determined by the standard Bethesda method.[4] For molecular genetic studies, DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-containing blood samples, by salting-out method. Initially, intron 22 inversion and then intron 1 inversion were screened using inverse shifting polymerase chain reaction (IS-PCR) and two multiplex PCRs, respectively. Following negative results for intron-1 and -22 inversions, PCR was performed to amplify the 26 *F8* gene exons. These reactions were performed on a normal control as well. DNA Sanger sequencing was performed, as previously described. For haplotype analysis, we used Haploview software (www.broad.mit.edu/mpg/haploview/). The IVS7 nt 27 SNP and BclI intragenic markers of *F8* gene were analyzed using the tetra-primer amplification refractory mutation system-PCR (ARMS-PCR) technique. Three extragenic short tandem repeat (STR) polymorphic markers, including DXSF8SU6, DXSF8SD1.6, and DXSF8SD15.9, were selected for linkage analysis. Capillary electrophoresis was performed by ABI 3130 Genetic Analyzer, and results were analyzed using GENEMAPPER

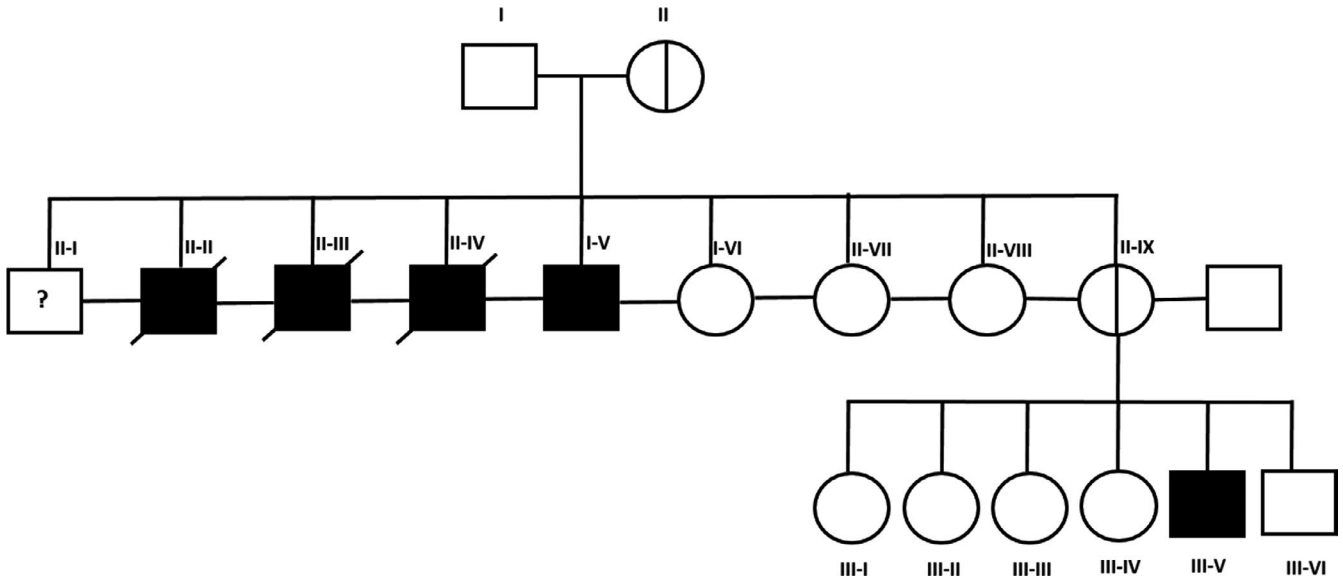

FIGURE 1 A family with hemophilia A and a high rate of morbidity. Index case (I-V) has severe hemophilia A (<1%) and high-titer inhibitor (2529BU). Three brothers (II-II, II-III and II-IV) of the patient had died due to bleeding. The mother and one sister (II-IX) of the patient was carrier with a son with severe hemophilia, while other sisters were unaffected. The only live brother of the case was not available for the molecular study, but he was asymptomatic. One uncle of the patient had died due to postcircumcision bleeding, while two others were asymptomatic

ID software v3.2. Multiplex-ligation-dependent probe amplification (MLPA) was used for confirmation of the large deletion of exon 1-25. The study was started on a 33-year-old Afghan patient with severe HA (FVIII coagulant activity (FVIII: C) less than 1%. HA was diagnosed in this patient, when he was 6 months old, at the Fatimid foundation in Pakistan. The patient suffered from severe gum bleeding and epistaxis, but the main reason for referral to the Fatimid foundation was a positive family history of HA. Four brothers of the patient suffered from severe HA, but died. The patient's uncle also had died due to postcircumcision bleeding. Treatment was hard to administrate due to distance and economic situation. Therefore, they received limited, irregular, on-demand therapy. Severe HA has led to the deaths of four sons, due to central nervous system (CNS) bleeding in two, gum bleeding in one, and severe postdental extraction bleeding in the last.

The family's last boy was referred to Iran for molecular study and further laboratory assays. It was confirmed with one-stage PT FVIII assay that the patient has severe HA. Unfortunately, the patient also has a high-titer inhibitor (2529BU). In the molecular study, we failed to amplify exons 1-25 of the patient, while these were done successful for the normal control. We suspected the deletion of these exons, and MLPA confirmed it. In fact, the patient has deletion of almost the entire *F8* gene (exon-1 to exon-25 deletion). The samples of three sisters and three nieces of the patient were available for molecular study. Indirect molecular study revealed that the patient's mother, sisters, and nieces were HA carriers. FVIII: C assay showed that the patient's nephew also had severe HA (FVIII: C < 1%).

The titer of inhibitor was increased after each FVIII concentrate infusion, and therapeutic response was absented. Due to limited therapeutic resources, lack of a therapeutic response, and the deaths of his brothers, the patient remains uncircumcised. Although CNS bleeding was the cause of death in two sons, other apparently minor bleeding—gum bleeding and postdental extraction bleeding—also caused death (Figure 1; Table 1).

The patients received different therapeutic products, including fresh frozen plasma and cryoprecipitate, as well as FVIII concentrates, from different companies.[1] This can decrease the efficiency of treatment and can provoke inhibitor formation. HA is caused by *F8* pathogenic variants, most commonly intron 22 inversion, which leads to severe HA. Gross gene defects are extremely rare in HA, responsible for ~35% of severe HA.[5,6] Several large deletions have been reported in these patients, but almost entire *F8* gene deletion (exon 1-to-25) has never been reported.[6,7] That patients with gross gene defects are more susceptible to inhibitor development has been noted in several studies.[8] This, along with exposure to FVIII concentrate from different companies, could be the main reasons for development of a high-titer, high-responder inhibitor.[9,10]

The present family is a unique example of the destructiveness of severe HA resulting from the largest reported *F8* gene deletion: a high rate of mortality without treatment easily and largely available, including bypassing agents in the presence of an inhibitor. All aspects should be considered in the management of patients with HA, including the type of *F8* pathogenic variants, therapeutic options,

TABLE 1 Characteristics of an Afghan family with hemophilia

Patient	Age (Y)	Age at diagnosis	Clinical presentations	Treatment	FVIII: C	Inhibitor	Molecular	Age at death (Y)	Cause of death
Case	38	6 M	Hemarthrosis gum bleeding Hematoma	On-demand	<1%	2535BU	Exon 1 to 25 deletion	—	—
Son-1	—	2 Y	NA	On-demand	<1%	ND	ND	18	CNS bleeding
Son-2	—	1 Y	NA	On-demand	<1%	ND	ND	15	Postdental bleeding
Son-3	—	1 Y	NA	On-demand	<1%	ND	ND	12	CNS bleeding
Son-4	—	1 Y	NA	On-demand	<1%	ND	ND	11	Gum bleeding
Nephew	5	6 M	Gum bleeding Hemarthrosis	On-demand	<1%	ND	ID	—	—

Abbreviations: FVIII: C, factor VIII coagulant activity; M, month; NA, not available; ND, not determined; Y, year.

severity of the disorder. Personalized hemophilia care programs should be considered, in order to decrease the adverse effects of HA and increase these patients' quality of life.

KEYWORDS

bleeding, deletion, hemophilia, inhibitor, mortality

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

A. Dorgalaleh designed the work, performed laboratory analysis, and wrote the manuscript. SH. Mousavi, S. Zeinali, M Jazebi, A. Dabbagh, SA. Mesbah-Namin, SMR. Hosseini, F. Zafarghandi Motlagh, and Y. Shiravand performed molecular and clinical studies. All the authors approved the submission.

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