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LETTER TO THE EDITOR

Factor XIII Deficiency in Western Afghanistan due to a Novel *F13A* Gene Mutation

Congenital factor XIII (FXIII) deficiency with an estimated incidence of one per 2 million is one of the rarest congenital bleeding disorders. Patients with severe congenital FXIII deficiency present umbilical cord bleeding as the most common clinical presentation (>80%), intracranial hemorrhage (ICH) as the main cause of death (~15% mortality), and recurrent miscarriages, with a frequency of ~100% in untreated women.[1,2] Due to the high rate of life-threatening bleeds, late diagnosis leads to fatal ICH in about one-third of the patients.[2] Early diagnosis and appropriate management of the disorder can alleviate or significantly decrease the rate of bleeds.[3] The disorder can be managed by fresh frozen plasma (FFP), cryoprecipitate, FXIII concentrate, and recombinant FXIII (rFXIII).[4] The incidence of the disorder is significantly higher in areas with a high rate of consanguinity such as Iran, India, Pakistan, and Afghanistan.[3,5] The present study reported the first family with congenital FXIII deficiency in western Afghanistan.

A neonate was presented to hospital with umbilical cord bleeding but without an abnormal bleeding history in the family, Afghan immigrants in Iran. At birth, the neonate experienced umbilical cord bleeding and delay in separation of the cord and was admitted to hospital. Initial examinations revealed no coagulopathy on routine coagulation tests. Physicians failed to diagnose a coagulopathy due to the scarcity of the disorder, and the normal results. The umbilical cord bleeding was managed by transfusion of blood components, but the disorder was not diagnosed. In the interval, the patient experienced several unusual bleeding episodes, including post-traumatic extracranial hemorrhage (ECH). At 2.5 years of age, the patient was referred to the hematology department with an abnormal bleeding history. Initially, the study was approved by the ethical committee of Tarbiat Modares University of Medical Sciences (IUMS) and written consent was obtained from all participants in accordance with the Declaration of Helsinki. At baseline, prothrombin time (PT) and activated partial thromboplastin time (APTT)-based assays were performed by STA compact automated coagulometer (Stago, Paris, France), and platelet count was carried out by Sysmex Autoanalyser (Sysmex KX-21 Haematology Analyser; Kobe, Japan). Following normal results in the first-line screening tests, clot solubility test, FXIII activity (Berichrom Factor XIII, Dade Behring, Marburg, Germany), and antigen assays (FXIII-A subunit by ELISA method) were performed on the neonate; samples from other family members were not available. The clot solubility test was positive and FXIII activity, and antigen levels were undetectable, revealing a severe FXIII deficiency (Table 1).

The patient was the second proband of the family with a positive consanguinity. No abnormal bleeding was observed in other family members. The patient immediately received Fibrogammin P (CSL Behring, Marburg, Germany) with a dose of 10 IU/kg every 4 weeks. Therapeutic response was excellent, without abnormal bleeding during the prophylaxis period. However, the patient's family could not afford continued Fibrogammin P. The patients received cryoprecipitate instead, but suffered a severe allergic reaction. Over the next two years, the patient received irregular Fibrogammin P prophylaxis. At 4 years of age, he was circumcised, but in spite of pre-operative administration of 40 IU/kg Fibrogammin P, the patient experienced severe prolonged postcircumcision bleeding and was hospitalized. The patient was managed again by Fibrogammin P and was discharged on the next day. Following abnormal results of the patient's clot solubility test, and undetectable FXIII antigen and activity levels, samples from all family members were collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant for molecular studies. To isolate genomic DNA, the blood was lysed using sodium dodecyl sulfate and proteinase K treatment of the buffy coat. The DNA was purified using phenol-chloroform and ethanol precipitation. Following extraction, genotyping for *F13A* gene mutations was performed by direct sequencing using ABI DNA Sequencer (Applied Biosystems, Foster City, CA, USA). This led to identification of a novel mutation in the *F13A* gene (c.563G > T, p.Trp188Leu) in homozygote state in the patient and heterozygote state in his parents, while the daughter was unaffected (Figure 1).

Multiple alignment revealed that tryptophan amino acid in position of 188, is conserved among human transglutaminase superfamily. Tryptophan 188 is located toward the surface of the FXIII protein between the catalytic core domain and the β-sandwich domain. Tryptophan forms a stable cavity between these domains. Substitution of tryptophan with leucine might be expected to lead to destabilization of the protein and early degradation of mutant FXIII.

Diagnosis of congenital FXIII deficiency is a challenge in Afghanistan due to the lack of a comprehensive coagulation laboratory, with only one period reported available for this country, whose diagnosis was performed in Iran.[6] The previous study revealed a relatively high incidence of the disorder with a high rate of mortality in

TABLE 1 Clinical and laboratory characteristics of patient with congenital factor XIII deficiency and family members

	Age	Sex	Clinical presentations	FXIII activity	FXIII A-subunit antigen	Mutation
Father	42	M	-	ND	ND	c.563G > T, p.Trp188Leu (Hetero)
Mother	39	F	Hem	ND	ND	c.563G > T, p.Trp188Leu (Hetero)
Proband 1	4.5	M	UCB, ECH, EP, Hem	<1%	<1%	c.563G > T, p.Trp188Leu (homo)
Sister	5.5	F	-	ND	ND	Nonmutant

Abbreviations: ECH, extracranial hemorrhage; EP, epistaxis; F, female; FXIII, Factor XIII, Hem: hematoma; M, male; ND, not determined; UCB, umbilical cord bleeding.

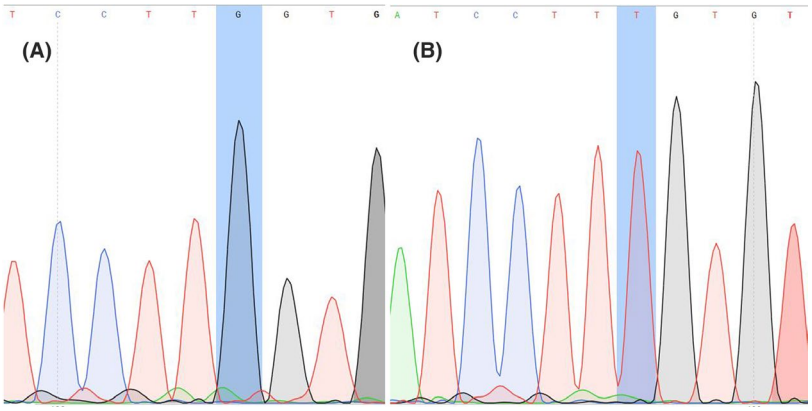

FIGURE 1 In non-mutant family members, nucleotide number 563 was cytosine (A), while in homozygote patients, this nucleotide was changed to T (c.563 C > T) (B). [Colour figure can be viewed at wileyonlinelibrary.com]

affected families in southwestern Afghanistan. All previous affected cases were of Baluch ethnicity, with the same mutation occurring in Iranian patients in southeast Iran (p.Trp187Arg, c.559T > C or according to HGVS: Trp188Arg, c.562T > C) associated with severe congenital FXIII deficiency.[6] It seems that, due to the founder effect, patients in these adjacent areas who are of the same ethnicity have the same FXIII deficiency-causing mutation, which differs from that in other parts of Iran. In the present study, the causative mutation occurred in the adjacent nucleotide at the same amino acid codon (c.563G > T, Trp188Leu). In addition to the Afghan mutation, a nonsense mutation at the same position (c.563G > A, Trp188Stop) was reported from Japan, also associated with severe FXIII deficiency.[7] The c.559T > C was observed in homozygote state in one per approximately 500 individuals and 3.5% heterozygotes in the area of Khash city in southeast Iran.[8,9] Congenital FXIII deficiency is accompanied by a high rate of morbidity and mortality, as previous studies on Iranian and Afghan patients have confirmed.[3,7] ICH and umbilical cord bleeding are the main causes of death among these patients. Late diagnosis leads to death in one-third of the patients due to ICH, but timely diagnosis and appropriate management can reduce fatal consequences.[3] Although diagnosis is a challenge worldwide, this issue is more profound in some developing countries such as Afghanistan with limited diagnostic and management facilities.[6] Since all reported cases from Afghanistan had mutations in the same codon, molecular diagnosis can be performed by a simple PCR-RFLP test or PCR sequencing of exon 4 of the *F13A* gene. This test can be used for carrier detection, prenatal diagnosis, and precise and rapid diagnosis that can significantly reduce the morbidity and mortality rate of the disorder and prevent further expansion of FXIII deficiency in Afghanistan, which Iran experienced for a considerable time.[9,10]

CONFLICT OF INTEREST

The authors have nothing to disclose.

AUTHOR CONTRIBUTION

A. Dorgalaleh designed the work and performed laboratory analysis, analyzed the data, and wrote the manuscript. SH Mousavi, M. Shams, S. Zeinali, and SA Mesbah-Namin performed molecular and clinical studies. D. Morant revised the manuscript. All the authors approved the submission.

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