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LETTER TO THE EDITOR

Small insertion (c.869insC) within F13A gene is dominant in Tunisian patients with inherited FXIII deficiency due to ancient founder effect

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Coagulation factor XIII (FXIII) is the last proenzyme that participates in the final stages of the clotting cascade, its main function is to stabilize the fibrin clot in the blood coagulation process. Plasma FXIII is a transglutaminase composed of two A subunits and two B subunits. The A subunit is responsible for the catalytic activity of plasma factor XIII, whereas the B subunit is thought to act as a carrier protein for the A subunit and to play a role in its protection and its stabilization in the circulation. The A and B subunits are the products of separate genes (F13A and F13B) that have been respectively mapped to chromosome 6p24–25 and 1q31–32.1 [1].

Congenital FXIII deficiency is a hereditary blood coagulation disorder, often characterized by an undetectable plasma FXIII activity and absence or decreased expression of the A subunit. Umbilical cord bleeding at birth, spontaneous intracranial haemorrhage and increased risk of miscarriage among pregnant women are often associated with FXIII deficiency. Diagnosis of this severe disorder might be delayed due to normal laboratory results upon screening, by prothrombin time, activated partial thromboplastin time and platelet count [1]. Over 300 cases of FXIII deficiency were reported in the literature since the first identification of this disorder (see http://www.f13-database.de/). Incidence of FXIII deficiency is estimated to be one patient per 2 millions in the general population. In Tunisia (a country with a population of 10 millions), 53 cases of FXIII deficiency have been identified since 1977. A total of 38 individuals out of these 53 died from fatal bleedings often including intracranial haemorrhage and some cases with post-abortion haemorrhage. In addition, the patients were unable to go to hospitals for a regular prophylactic replacement therapy.

Globally, most of the cases were due to mutations in the A subunit gene that consists of 15 exons spanning over 160 kb. However, a few cases with mutation in FXIII-B gene have been reported [2], FXIII gene mutation databases at http://www.hgmd.cf.ac.uk and http://www.med.unc.edu/isth/mutations-databases. In Tunisia, only one mutation in the subunit A was identified until now.

In this study, we report molecular characterization of FXIII subunit A deficiency in 13 patients belonging to 10 Tunisian families. All subjects gave informed consent according to a protocol approved by the Local Research Ethics committee.

The subjects are the progeny of consanguineous marriages. All individuals suffered from umbilical cord bleeding few days after birth and experienced severe bleeding episodes, such as intra-abdominal haemorrhages and haemarthroses. Two women suffered from recurrent spontaneous miscarriages (Table 1), the patient 8 had three pregnancy and the patient 12 had five pregnancy, but both of them were not able to maintain pregnancy. They all received fresh frozen plasma to treat the bleeding.

The activity of plasma FXIII A was determined by a fluorescence assay using dansylcadaverine. Antigen levels of FXIII A and B were measured by Laurel's method (using specific antibodies). All patients had undetectable FXIII activities (<1%), which revealed a major FXIII deficiency.

In these 13 patients, all exons of the A subunit gene were amplified with primers, allowing the analysis of the intron–exon boundaries.

The PCR products of each exon showed the expected size in all patients. Following sequencing

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Table 1. Clinical phenotype information of probands with FXIII A subunit deficiency.

Family	Patient	Sex	Date of birth	Clinical symptom	Wound healing	Intracerebral bleeding	FXIII A activity (%)	FXIIIA Antigen	FXIII B Antigen (%)
K	1	M	1968	Umbilical bleeding, subcutaneous haematomas, Epistaxis			<1	Undetectable	30
M	2	M	1986	Umbilical bleeding,			<1	Undetectable	38
	3	F	1987	Umbilical bleeding, echymoses	X		<1	Undetectable	50
	4	F	1994	Umbilical bleeding, subcutaneous haematomas	X	X	<1	Undetectable	50
N	5	F	1982	Umbilical bleeding, mouth haemorrhage, subcutaneous haematomas	X		<1	Undetectable	45
S	6	F	1994	Umbilical bleeding peritoneal haematomas		X	<1	Undetectable	38
SM	7	F	1985	Umbilical bleeding, bleeding after trauma	X		<1	Undetectable	48
R	8	F	1959	Umbilical bleeding, mouth haemorrhage, 7 miscarriage		X	<1	Undetectable	60
BA	9	F	1975	Umbilical bleeding, subcutaneous haematomas, peritoneal haematomas	X		<1	Undetectable	65
SK	10	F	1974	Umbilical bleeding, intramuscular Bleeding	X	X	<1	Undetectable	36
T	11	M	1963	Umbilical bleeding, subcutaneous haematomas	X		<1	Undetectable	47
	12	F	1969	Umbilical bleeding, epistaxis, 5 miscarriages	X	X	<1	Undetectable	50
BR	13	F	1977	Umbilical bleeding, hemarthrosis, epistaxis	X		<1	ND	ND

ND, not determined.

of the amplified exons, the same mutation in the FXIII A gene was revealed in all Tunisian patients. This mutation consists in a C insertion in exon 7 (c.869insC; sequence access number NM 000129.3, GI 119395708). This insertion results in generation of seven altered amino acids, followed by a termination codon downstream at position 260 (ASP260X; NP 000120.2, GI 119395709), predicting a truncated FXIII A polypeptide that lacks most of the catalytic core domain. This truncated polypeptide is likely to be unstable. This mutation was present in the homozygous state in all patients and in the heterozygous state in their parents. This mutation was previously reported in one Tunisian patient [3].

The identification of the same mutation among 10 families living in the southern and northern regions of Tunisia suggests the existence of a founder effect. To prove the presence of this founder effect, we analysed six common SNPs within the FXIII A gene, including Val34Leu (c.103G>T), Pro331Pro (c.996A>C), Pro564Leu (c.1697C>T), Glu567Glu (c.1707A>G), Val650Ile (c.1951 G>A) and Glu651Gln (c.1954 G>C). The result demonstrated the same haplotype Val34-Pro331-Pro564-Glu567-Val650-Gln651 carried by all our patients, which supports our hypothesis.

Moreover, the c.869insC mutation has not been described in other populations and it seems to be specific to the Tunisian population. The high consanguinity rate in Tunisian population (estimated at 33% and could reach 86% in some rural communities) would cause the high FXIII deficiency's prevalence in Tunisia.

Based on information given by families' members, registers of births, marriages and deaths, we have drawn the ascendant genealogy for the eight families originating from Sned, a village close to Gafsa in southern Tunisia (Fig. 1). More investigation about these families and the two other families originating, respectively, from Northern Tunisia and from Gettar, a village near Gafsa, has been undertaken using historical data and public records [4,5]. Hence, we were able to show that all these families are descendants of one man named Hammam, who came to Tunisia with the Bani hilal invasion around 1050. Following historic data, this tribe came from Hidjaz (Kingdom of Saudi Arabia) [5]. Descendants of Hammam have been well documented through the genealogy presented in Fig. 2. Hammam's sons (Idriss I and Rabii) were at the origin of three lineages to which family BR, the group of the eight families (SK, S, BA, M, SM, N, K and R) and family T, respectively, belong. This genealogy allowed us to suggest the link between all the studied Tunisian

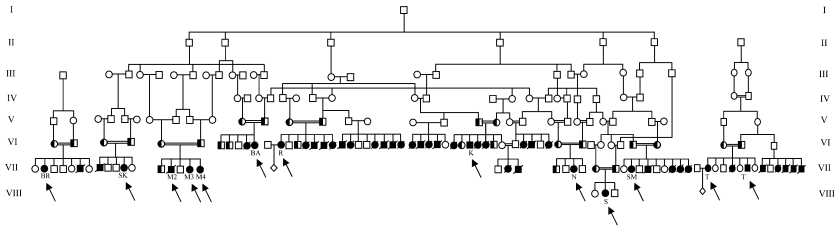

Fig. 1. Pedigree of the studied families. Latin numbers represent the generations. Generations from one to eight are determined with information given by members of the families, registers of births, marriages and deaths. ○: female. □: male. • ■: individuals who are homozygous for FXIII deficiency and therefore affected by the disease. ◐◧: semi-filled symbols: individuals who are heterozygous and therefore only carriers of the disease but not affected. • ■: died individual. The 13 patients with severe FXIII deficiency are indicated with arrows.

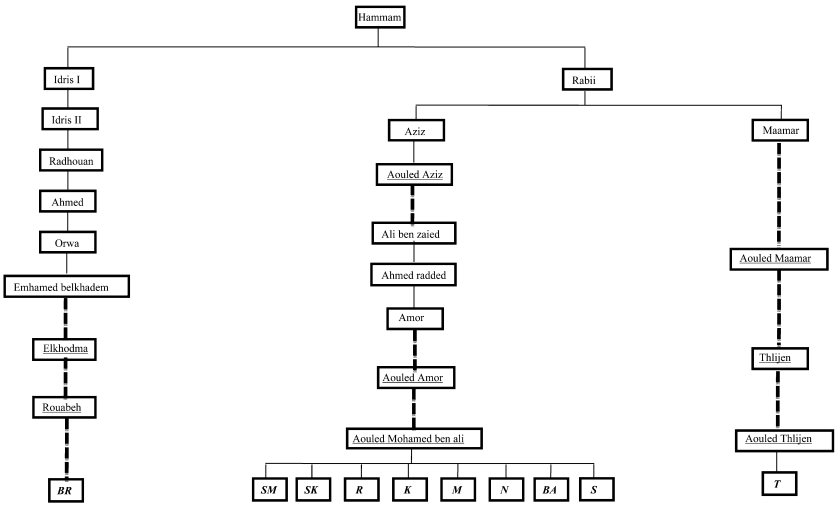

Fig. 2. Common ancestor of the 10 families. This figure shows the Relationship between families belonging to the hammam tribe and to whom our families with FXIII deficiency are related. In this simplified representation: names subligned represent a group of families with common ancestors, continued lines represent one generation, discontinued lines represent generations. Italic letters correspond to the names of the studied families.

families with FXIII deficiency, which might be attributed to a common ancestor: Hammam (Fig. 2).

This founder effect simplifies the molecular diagnosis of FXIII deficiency. Indeed, the main objective of molecular characterization of the deficit of FXIII in the Tunisian population is to allow precise diagnosis of patients and to allow antenatal diagnosis. To this aim, the exon 7 will be the first region to be sequenced for identification of the c.869ins C mutation, in the concerned families.

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Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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