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ORIGINAL ARTICLE

Congenital factor XIII deficiency caused by two mutations in eight Tunisian families: molecular confirmation of a founder effect

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Abstract Inherited factor XIII (FXIII) deficiency is a rare bleeding disorder characterized by an umbilical bleeding during the neonatal period, delayed soft tissue bruising, mucosal bleeding spontaneous intracranial hemorrhage, and soft tissue hemorrhages. Congenital FXIII deficiency is an autosomal recessive disorder, usually attributed to a defect in the FXIIIA and B subunits coding, respectively, by *F13A* and *F13B* genes. The aim of this study was to determine the molecular defects responsible for congenital factor XIII deficiency in eight Tunisian families. Molecular analysis was performed by direct DNA sequencing of polymerase chain reaction amplified fragments spanning the coding regions and splice junctions of the FXIIIA subunit gene (*F13A*) in probands and in families' members and compared with the reported sequence of this gene. In all patients, FXIIIA activity was undetectable and the FXIIIB was within the normal range. Direct sequencing of the *F13A* gene in all probands showed two mutations: the c.869insC mutation found in eight patients and the c.1226G>A transition found in only one. We also confirmed the presence of a founder effect for the first frequent mutation by using two microsatellite markers, *HUMF13A01* and a generated ployAC marker (*HUMF13A02*). We describe here molecular abnormalities found in nine Tunisian probands diagnosed with FXIIIA deficiency. The identification of the founder mutation and polymorphisms allowed a genetic counseling in relatives of these families, and the antenatal diagnosis is now available.

Keywords Factor XIII deficiency · Mutations · Polymorphisms · Founder effect · c.869 insC · c.1226G>A

Introduction

Congenital factor XIII (FXIII) deficiency is characterized by umbilical cord bleeding at birth, spontaneous intracranial hemorrhage, soft tissue hemorrhages, poor wound healing in women and spontaneous abortions [1]. In plasma, coagulation FXIII pro-enzyme circulates in the form of an A2B2 heterotetramer composed of two catalytic A subunits bound to two carrier B subunits [2]. Intracellular FXIII is found as a homodimer of two A subunits (A2) [3]. The A-subunit is composed of 731 amino acids and consists of an activation peptide and four distinct domains [4, 5]. The B-subunit is composed of 640 amino acids which form 10 tandem repeats [6]. Inherited FXIII deficiency (OMIM: +134570, +134580) is known as a rare autosomal recessive disorder affecting approximately one out of one to three million people [7]. This incidence rises in the south of the Tunisia to four cases for a million owing to the strong consanguinity [8]. Based on genotype, there are two types of FXIII deficiency: FXIIIA subunit deficiency (FXIIIA), when mutations affect the *F13A* gene [9], and more rarely (<5% of all cases), FXIIIB-subunit deficiency (FXIIIB), when mutations affect the *F13B* gene [10]. Both types result in absence of FXIII catalytic activity in plasma.

The genetic basis of FXIIIA deficiency has been studied in a number of cases and appears to be highly heterogeneous with at least more than 60 identified mutations [11].

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The gene coding for the FXIIIA subunit (*F13A*) is located on chromosome 6p24–25, spanning 160 kb and consists of 15 exons interrupted by 14 introns encoding a mature protein of 731 amino acids [12]. FXIIIA is divided into the activation peptide, b-sandwich, catalytic core, b-barrel 1, and b-barrel 2 [13]. Crystal structure of the A subunit revealed a catalytic triad in the central core domain formed through hydrogen bond interactions between Cys314, His373 and Asp396 [4].

Patients and methods

Patients and families

Nine patients form the south of Tunisia with a severe FXIIIA deficiency were explored in this study. These patients (three males and six females) were clinically investigated between 1985 and 2009. Clinical features were reported in a previous study [8]. Factor XIII subunit A activity was less than 1% in all the studied patients, whereas the subunit B activity ranged between 32% and 50% [8].

Methods

DNA extraction

Peripheral blood samples were obtained from all the patients and families members after informed consent and with the approval of the appropriate Ethic Committee. Genomic DNA was purified from peripheral blood leucocytes according to the phenol-chloroform protocol [14].

Mutation analysis

Following DNA extraction, the coding regions and intron-exon boundaries of the *F13A* gene encoding the A subunit were amplified by polymerase chain reaction (PCR) using generated primers covering the entire coding region (Table 1). PCR reaction was carried out in a GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The purified amplicons were directly sequenced using a Dye Terminator Cycle Sequencing Kit with an ABI sequence analyser 3100 avant (Perkin Elmer, Norwalk, CT, USA) according to the manufacturer's recommendations.

Microsatellite typing

For the haplotypic analyses, we screened two microsatellite markers (Table 2). The *HUMF13A01* (AAAG)n occurs in the 5′ flanking region of the *F13A* gene and reported as highly polymorphic STR [15]. The second marker: poly AC (*HUMF13A02*) was newly generated from the genomic sequence of *F13A* gene using Tandem Repeats Finder Software (http://tandem.bu.edu/trf/trf.html) and it is located in intron 8 of the *F13A* gene. These markers were typed in all available family members. After PCR amplification, amplified products were separated on 10% polyacylamide gel, stained with ethidium bromide and visualized under ultraviolet light. A haplotype co-segregating with the disease was derived from the segregation of markers within all pedigrees. The sizes of the PCR products of the co-segregating microsatellite markers were compared between affected members of the nine families. To establish the correspondence between genotyping results and repeat number, some PCR products (homozygous) were sequenced for each microsatellite marker.

Sequence alignment

The sequence alignment was performed using the Clustal W program (http://bioinfo.hku.hk/services/analyseq/cgi-bin/clustalw_in.pl).

Results

In this study, we screened the *F13A* gene in nine patients (three males and six females) belonging to eight unrelated consanguineous Tunisian families who have severe FXIIIA deficiency. All the cases had undetectable plasma FXIII activities (<1%) and absence or decreased expression of the A subunit. Umbilical bleeding was common and only two patients had intracranial bleeding.

F13A gene mutational analysis

All exons and intron-exon boundaries of the *F13A* gene were sequenced and the c.869insC mutation was detected in eight patients (Fig. 1a) whereas the c.1226G>A mutation was detected in only one (Fig. 1b). The c.869insC is a frameshift mutation occurring in exon 7 of *F13A* gene and leading to a null allele changing the 8 amino acids downstream the cytosine insertion which causes a premature termination of translation. The c.1226G>A transition in exon 10 of the *F13A* gene leads to the replacement of an arginine residue with glutamine at position 408 of the protein (p.R408Q). Verification of transmission of these mutations was done for all families. These variants were absent by direct sequencing in 100 Tunisian control chromosomes. These healthy controls were selected from the same region of the studied patients.

The alignment of the F13A proteins in several species showed that our mutations affect a highly conserved

Table 1 PCR primers used to amplify the coding sequence of *F13A* gene

Name	Genomic position	Forward primers	Reverse primers	PCR product size
F13Aex2	4257..4404	5′TTTGGAATCCTTGTCTCAAATG3′	5′TACCTGCAGGGAAGAGGG3′	298 Bp
F13Aex3	17405..17593	5′CCTTCTTCAGGGAAAATAATCTTG3′	5′ACTGTGCCTGTACCCACCTC3′	355 bp
F13Aex4	56137..56388	5′GATTACTCCTGTCCCTTCAGC3′	5′AAGCGATCCTCCCATCTTG3′	460 bp
F13Aex5	72018..72136	5′ATGCTTGGTGAAACAAAGGG3′	5′CGCAGTTGTCTTTATGAGTCC3′	434 bp
F13Aex6	74528..74635	5′GGAAACACACATTTCTAAACATTAGG3′	5′AAGTGTAAGTCATCATTTCAGCAG3′	320 bp
F13Aex7	98087..98261	5′CCATGCATAGCCTGGAGTAG3′	5′AACAGGGGCTGCTATGTCTC3′	343 bp
F13Aex8	100778..100916	5′CAGGAAGAAAGTCCCCTACAAG3′	5′TTGAGTCTATCTTGTGGTAAATGTG3′	307 bp
F13Aex9	125625..125728	5′GGCCGTCACTTCTTGATCTC3′	5′TCCCACACATCAAATGCAAG3′	260 bp
F13Aex10	127066..127154	5′TGCCATGAATCTTGCAGTATC3′	5′CCTGGAAAACTTAGAAACAACAAC3′	258 bp
F13Aex11	140810..140963	5′CGACTGCTCTATGGACAGTTAAAG3′	5′TCATCTCTGAGTGACAATGAATAAG3′	309 bp
F13Aex12	148084..148371	5′TGCATCCATTAATGTGACTTTC3′	5′AACGGGCATTAACACCTAGC3′	434 bp
F13Aex13	155336..155496	5′AGGGGTAGGGAAACTTGGAG3′	5′TTTGAGCAGGACATTCATTCAC3′	358 bp
F13Aex14	171005..171141	5′TCACACTGGTTCCATTGCC3′	5′TGGGGAGCAGATCTATGTTTG3′	358 bp
F13Aex15	177182..177335	5′GGGGCATAACTTGCTGAATC3′	5′ATGTGGGATCTCCACTCTG3′	458 bp

domain responsible for the catalytic activity of transglutaminase (TGase) family (Fig. 1c, d)

Microsatellite typing

According to the prevalence of the c.869insC mutation among our group of patients (eight of nine) and since the seven studied families sharing the same mutation c.869insC are all originated from south Tunisia, we hypothesized that high frequency of the c.869insC may represent a founder effect. We performed haplotype analysis in all families' members using the *HUMF13A01* and the poly AC generated *HUMF13A02* markers (Fig. 2c) and also the known SNPs located in the *F13A* gene. A founder effect is expected to result in sharing of allelic sequence polymorphisms in the vicinity of this mutation (linkage disequilibrium due to a common ancestor). We examined at first *HUMF13A01* marker within the 5′UTR (-2775/ATG) of *F13A* gene and found that a rare 200 bp allele containing four (AAAG) repeats was shared by the eight patients harboring the same mutation c.869insC (Fig. 2). In contrast, the patient with the c.1226G>A mutation displayed differently sized allele (206 bp) for the same marker as the disease allele in the other affected patients containing five (AAAG) repeats instead of four (Fig. 2). Furthermore, the 200 bp allele was associated to a two-base (GT) deletion polymorphism downstream the four (AAAG) repeated sequence (Fig. 2). This deletion was found in all patients with the c.869insC mutation suggesting that the 200 bp allele cosegregate with the insertionnal mutation c.869insC which is in favor of a founder effect in the seven Tunisian families (Fig. 3).

To confirm the presence of a founder allele for the c.869insC mutation, we generated the new *HUMF13A02* marker (g.161401/ intron 8 of *F13A* gene) which revealed informative (more than four different alleles in 50 tested chromosomes: data not shown) and allowed to define the disease haplotype. Patients sharing the c.869insC mutation showed a homozygous 204 bp allele with 22 (AC) repetitions (Fig. 2a), whereas the patient with the c.1226G>A mutation showed another homozygous 208 bp allele containing 24 (AC) repetitions (Fig. 2b).

Genotyping using the two microsatellite markers confirmed that the c.869insC mutation has been transmitted by a common ancestor. To further strengthen this association, we analyzed known SNPs which showed a common haplotype in all families. However, the SNPs data was less informative than microsatellites analysis because the SNPs were less polymorphic (Fig. 3). Together, these results strongly suggest that a founder effect is responsible for the increased frequency of c.869insC mutation in familial congenital FXIII deficiency in Tunisia.

Table 2 Polymorphic microsatellite markers within *F13A* gene

Marker name	Primer sequence	Repeat motif
HUMF13A01 F HUMF13A01 R	5′AGCCTTTGCAACAGAGCAAG3′ 5′GCCCCAAGGAAGATGAGTAA3′	(AAAG)n
HUMF13A02 F HUMF13A02 R	5′TTTTCTCTGCCTTCCCATGT3′ 5′CCCCCAGTGCAGTGTTTTAT3′	(AC)n

Fig. 1 Sequence chromatograms in controls and patients of the c.869insC insertion (**a**) and the c.1226G>A mutation (**b**). Nucleotide variations are indicated by an *arrow*. **c** Alignment of the mutated Asp260X F13A protein with transglutaminase from different species showing the conservation of the catalytic region. **d** Alignment of the mutated Arg408Glu F13A protein with transglutaminase from different species showing the conservation of the amino-acid 408; the mutated amino acid is *framed*

Fig. 2 Sequence chromatograms of the microsatellites markers *HUMF13A01* and *HUMF13A02* for patients with the two identified mutations. **a** All patients (P1–P7) carrying the c.869insC mutation showed the presence of 200 bp allele containing 4 (*AAAG*) followed by a two-base (*GT*) deletion polymorphism for *HUMF13A01* (two-base (*GT*) deletion was indicated by an *arrow*). This allele was associated to a 204 bp allele with 22 (*AC*) repetitions polymorphism with the HUMF13A02 marker. **b** Patient P8 carrying the c.1226G>A mutation showed two different alleles: 206 bp allele containing five (*AAAG*) repeats with *HUMF13A01* associated to a 208 bp allele containing 24 (*AC*) with *HUMF13A02* marker. **c** Position of the *HUMF13A01* and *HUMF13A02* microsatellite markers on *F13A* gene

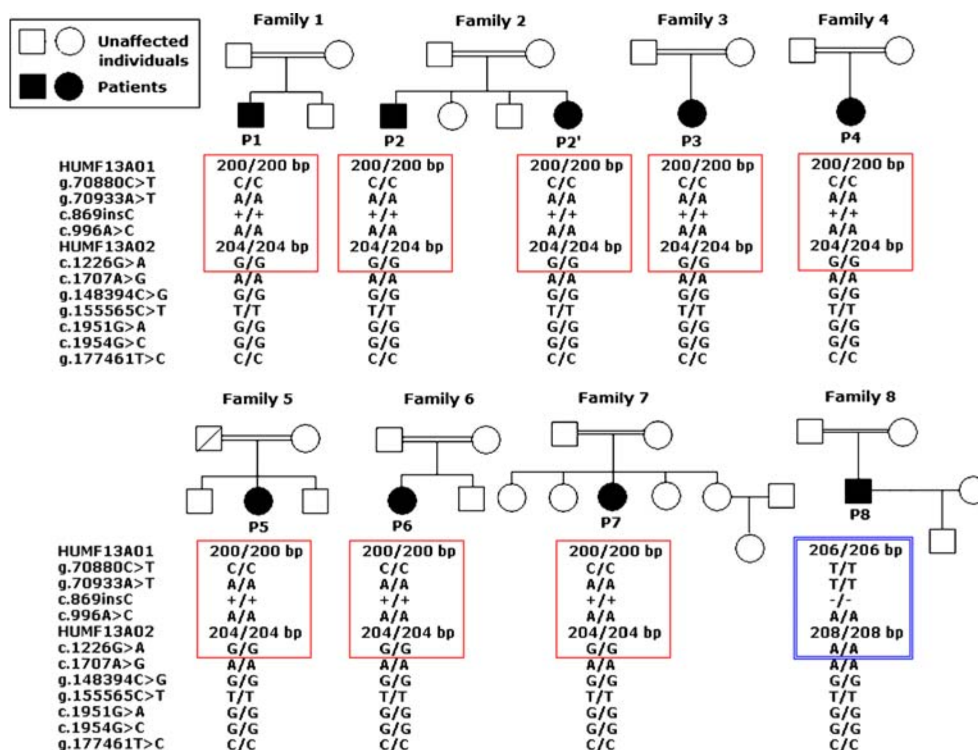

Fig. 3 The pedigrees of the eight Tunisian families showing the inheritance of the c.869insC or the c.1226G>A mutations. The informative haplotypes containing mutations are *framed*. We note two different haplotypes: a founder haplotype for families 1 to 7 having the c.869insC mutation and a different haplotype for family 8 carrying the c.1226G>A mutation (*double framed*). The part of haplotypes *not boxed* is non-informative for all patients and their relatives. +/− Presence/absence of the c.869insC mutation

Discussion

In this report, we studied nine patients belonging to eight unrelated consanguineous Tunisian families with severe FXIIIA deficiency. Mutation analysis of *F13A* gene revealed the presence of the homozygous c.869insC mutation in exon 7 in eight out of the nine patients. This mutation changed the 8 amino acids next to the insertion leading to a premature termination codon (ASP260X). The predicted mutated protein is a truncated FXIIIA polypeptide that lacks most of the catalytic core domain. Indeed, bioinformatics comparison between wild and mutated proteins revealed that our mutation leads to a truncated protein of 297 instead of 731 amino acids for the wild one.

Up to now, more than 60 different pathogenic mutations have been reported in *F13A* gene (http://www.f13-database.de). The c.869insC mutation is specific for Tunisian population. Indeed, this mutation was previously reported for the first time in an Arabo-Israeli family originated from Tunisia [16] and recently, El Mahmoudi et al. described the same mutation in 13 patients belonging to ten families living in the southern and northern regions of Tunisia [17].

In addition to the first mutation, we identified the c.1226G>A transition in only one patient. This mutation was also reported in patients from Yorkshire (UK) [18]. It changes an arginine residue to a glutamine at position 408 of the protein (p.R408Q). Since glutamine is a neutral polar amino acid, the Arg408Gln substitution may probably induce a conformational change around the catalytic triad, which in turn would grossly affect the activity of the protein [19].

Since the c.869insC mutation has not been described in other populations, it seems to be specific to the Tunisian population. According to such finding and in agreement with the prevalence of this mutation reported in our study and the previous ones [17], we demonstrated that a clustering of c.869insC mutation in seven Tunisian families is due to a common founder ancestor. In fact, the analysis of two microsatellite markers *HUMF13A01* and generated *HUMF13A02* with SNPs within the *F13A* gene revealed a common haplotype carried by the patients sharing the same c.869insC mutation. The remaining patient with c.1226G>A mutation showed different alleles constituting a different haplotype.

In addition, the seven families carrying the c.869insC mutation are originated from southern Tunisia (Sidi Bouzid and Gabes). Such rural communities are characterized by a high consanguinity and endogamy rates which supports our findings. However, the eighth family is originated from central Tunisia (Sfax).

Evidence for a founder effect has been also reported in previous studies [7, 20–22]. In our report, we provided a molecular confirmation of the founder ancestor for the c.869insC mutation recently reported by El Mahmoudi et al [17] since the SNPs used in this previous study are not sufficiently informative in Tunisian population as shown by our haplotypes (Fig. 3).

Moreover, the 200 bp allele of *HUMF13A01* marker was found to be associated with a two-base (GT) deletion polymorphism located just one base downstream of the repeated sequence. Our result was in agreement with those of

Hammond et al. [23]. Indeed, in all ethnic groups, the deletion allele was always found in association with the four repeats allele. On the other hand, the five (AAAG) was observed in association with the c.1226G>A constituting another allele with GT insertion [20]. The distribution of the deletion/insertion polymorphism in Caucasians, Africans, and Asians suggests that the insertion allele is ancestral and that the deletion event occurred more recently on a four repeat background. The complete association between the deletion and the four-repeat allele suggests that the *HUMF13A01* STR alleles are mutationally stable and have most likely arisen only once during human evolution [20].

In conclusion, we reported two mutations: c.869insC and c.1226G>A in *F13A* gene in eight consanguineous Tunisian families with severe FXIIIA congenital deficiency. We had also confirmed the presence of a founder ancestor haplotype co-segregating with the common c.869insC mutation. The identification of this “founder ancestor mutation” and polymorphisms allowed a genetic counseling in relatives of these families and the antenatal diagnosis is now available.

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