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Molecular analysis in two Tunisian families with combined factor V and factor VIII deficiency

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Summary. Combined factor V (FV) and factor VIII (FVIII) deficiency (F5F8D) is a rare autosomal recessive disorder caused by mutations in LMAN1 or MCFD2 genes which encode proteins that form a complex involved in the transport of FV and FVIII from the endoplasmic reticulum to Golgi apparatus. We report two novel mutations in MCFD2 gene and one recurrent mutation in LMAN1 gene that caused combined FV and FVIII deficiency in two unrelated Tunisian Muslim families. For the first family two patients were homozygous for a new missense mutation Asp81His in exon 3 of MCFD2 and heterozygous for a second new missense mutation Val100Asp in the same exon. Replacement respectively of the hydrophilic Asp residue with hydrophobic positively charged His and of the hydrophobic neutral Val residue with the Asp residue most likely disrupts the MCFD2–LMAN1 interaction, thus leading to the disease phenotype. For the second family a reported Arg202X mutation in exon 5 in the LMAN1 gene was identified in the homozygous state.

Keywords: combined FV and FVIII deficiency, F5F8D, LMAN1, MCFD2, mutation, Tunisia

Introduction

Combined factor V (FV) and factor VIII (FVIII) deficiency is inherited in an autosomal recessive manner, described in 1954 by Oeri *et al.* [1]. F5F8D is characterized with a mild-to-moderate bleeding tendency. Easy bruising, epistaxis and gum bleeding problems happen after surgery; dental extraction and trauma; menorrhagia and postpartum haemorrhage are also commonly seen in affected patient. Other types of symptoms can occur, including hemarthrosis and muscular haematomas. Excessive bleeding after circumcision was also reported [2,3].

Two genes are responsible for this disorders, LMAN1 and MCFD2 mapped to chromosomes 18 (18p21) and 2 (2p16.3) respectively [4,5]. The LMAN1 gene spans 29 kb and contains 13 exons, whereas the MCFD2 gene consists of 4 exons and is 19 kb in length [6]. In the normal state, the two proteins encoded by LMAN1 and MCFD2 form a cargo receptor complex that transports FV and FVIII newly synthesized in the ER to the Golgi apparatus [7]. Absence of the complex LMAN1/MCFD2 decreases the secretion of FV and FVIII, causing low levels between 5% and 30% respectively [7,8], of these two factors in the plasma. About 70% of mutations were identified in the LMAN1 gene and 30% in the MCFD2 gene [9,10]. In these reports, two novel mutations, namely Asp81His and Val100Asp both in exon 3 of MCFD2, and a reported Arg202X mutation in exon 5 in LMAN1 gene in two Tunisian unrelated Muslims families, are described as a cause of combined FV and FVIII deficiency.

Patients and methods

Patients

Three patients and six unaffected family's members from two unrelated Tunisian Muslims families were studied. Patients had been previously diagnosed on

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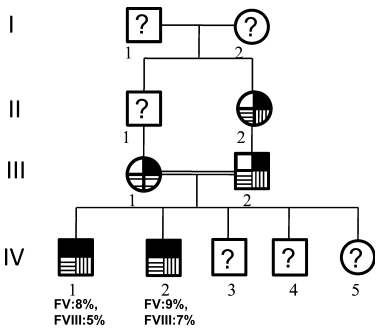

Fig. 1. Family A pedigree. □, Asp 81; ■, His 81; ▤, Val 100; ▥, Asp 100, ?, not determined.

the basis of a clinical bleeding tendency and low levels of FV and FVIII [3].

Methods

Informed consent was obtained from the patients and their available family members for the drawing of blood samples and for genetic studies.

DNA analysis. To determine the genetic defect, genomic DNA was extracted from peripheral blood by phenol chloroform method. All the exons and exon-intron boundaries of LMAN1 and MCFD2 genes were PCR amplified as described previously [11]. The amplicons were sequenced in both directions using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) and analysed using the software (Sequencing Analysis, ABI, Perkin-Elmer Applied Biosystems).

Result and discussion

For the first family, family A, direct sequencing of all amplified fragments allowed us to identify two novel genetic defects in exon 3 in MCFD2 gene, whereas null mutation was identified in LMAN1 gene. The first mutation was: g.241 G>C, leading to the Asp81His missense substitution (GAT → CAT), and the second missense mutation was: g. 289 T>A causing the Val100 His (GTC → GAC). The Asp81His was found in the homozygous state in the two brothers, offspring of a consanguineous marriage (patient A: IV: 1 and patient A: IV: 2; Fig. 1), whereas the Val100 His was found in the heterozygous state. The mother was found to be heterozygote for the Asp81His

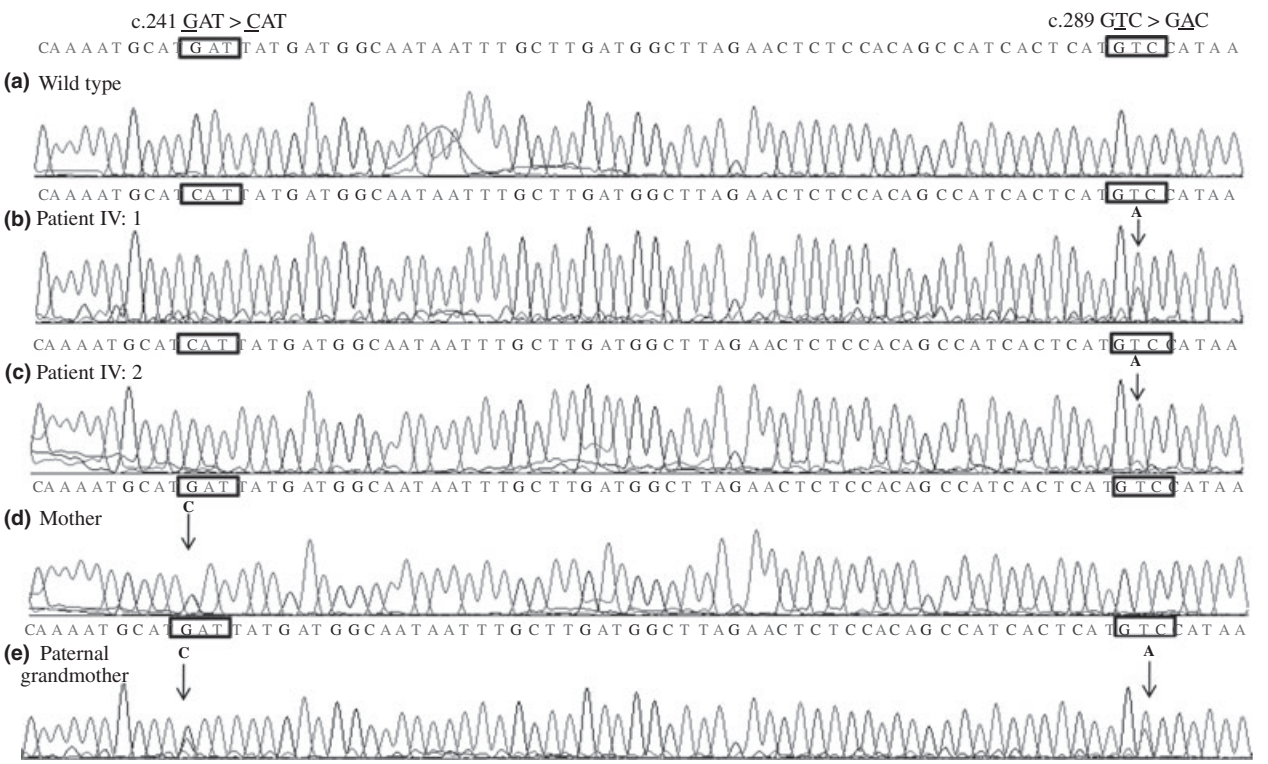

Fig. 2. DNA sequence surrounding nucleotide 241 and 289 of MCFD2 exon 3 in first family. (a) DNA sequence of the wild type. (b and c) DNA sequence of patient IV: 1 and patient IV: 2, they are homozygous for c.241 GAT>CAT which cause Asp81His and heterozygous for c.289 GTC>GAC which cause Val100 His. (d) DNA sequence of the mother, a heterozygous for c.241 GAT>CAT. (e) DNA sequence of the paternal grandmother a heterozygous for c.241 GAT>CAT and for c.289 GTC>GAC.

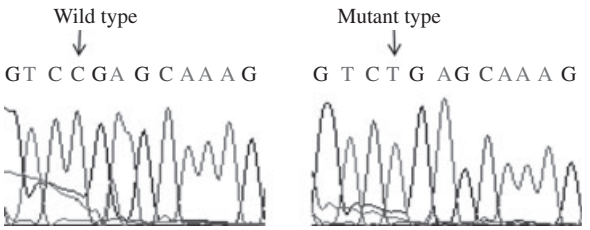

Fig. 3. DNA sequence showing the nucleotide substitution (c.C 604>T) in exon 5 of LMAN1 gene in the second family.

mutation; the paternal grandmother was found to be compound heterozygote for the Asp81His and the Val100His (Fig. 2). Therefore, the Asp81His was carried out from the two parents and the Val100His was carried out only from the father. As the paternal grandmother is healthy, it appears that both mutations belong to the same allele of the MCFD2 gene. Furthermore, one of the mutations occurred between generation I and generation II. Necessarily this would be the Asp 100 mutation occurring on the background of the His 81. It seems most likely that the disease causing mutation is in fact the His 81 and the second mutation could be a clinically silent change. To confirm this interpretation more investigation about mutated protein expression is needed.

The novel mutations have not previously been reported and genotype analysis of 55 healthy controls revealed only the wild type sequence at these positions, hence there are no common polymorphisms and they occurred at amino acid residues highly conserved in all known vertebrate suggesting important structural or functional roles for these residues.

For the second family, family B, sequencing revealed a reported nonsense mutation in the homozygous state in exon 5 in the LMAN1 gene in patient (Fig. 3): g.604 C>T nucleotide in the amino acid Arg 202 generating a stop codon [Arg202X (CGA → TGA)] also identified in both Iranian and Japanese populations [11,12]. The mutation was identified in the heterozygous state in both parents and was not detected in her sister (Fig. 4). Two polymorphisms [G351 → A in exon 2 and del GT (5 → 12) in intron 4] were also identified in the homozygous state in this patient. No mutation was detected in the MCFD2 gene. In the case of the Arg202X mutation identified in our patient, because no information about the polymorphisms associated with the Arg202X mutation in other populations was available, we could not confirm if it is a founder mutation.

F5F8 deficiency is a rare autosomal recessive disorder in which patients present low levels of FV and FVIII between 5% and 30%. This disorder is attributed to a defect in the LMAN1 gene or

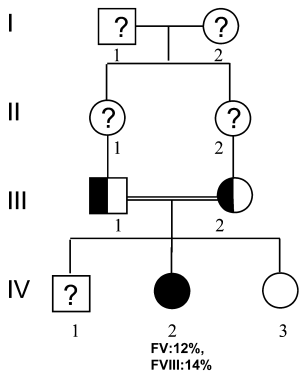

Fig. 4. Family B pedigree. ◧, Heterozygote for the c.604 C>T mutation; ◐, heterozygote for the c.604 C>T mutation; ●, homozygote for the c.604 C>T mutation; ○, health member; ?, not determined.

MCFD2 gene. The most mutations described, in LMAN1 gene, are nonsense mutations. Those identified in the MCFD2 gene mutations are missense and nonsense mutations. The five missense mutations (Asp81Tyr, Asp89Ala, Asp122Val, Asp 129Glu and Ile136Thr) reported in MCFD2 to date change highly conserved amino acids in one of the two EF-hand domains (The EF hand is a helix-turn-helix structural analysis found in a large family of calcium-binding proteins) [13].

The presence of consanguinity between the parents in these two families played an important role in increasing the appearance of this recessive disease, whereas a family history of bleeding was not found, which is in agreement with literature.

Currently, in Tunisian population, only the founder mutation [transition from a thymidine (T) to cytosine (C) in the donor site of intron 9] in the LMAN1 gene has been reported among the Jews of Djerba Tunisians [14]. We report here the first cases, in Muslim community, with defect in MCFD2 gene and LMAN1 gene.

Hundreds of families living with combined deficiency of FV and FVIII has been described, half of which come from the Mediterranean (Italy, Israel, Algeria, Tunisia, Turkey) and the other half being divided between the North America and South America, Japan and India [15]. The genetic study of combined FV and FVIII deficiency identifies genetic abnormalities and the mechanisms responsible for this deficit.

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Disclosures

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

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