Identification of a novel mutation in the MCFD2 gene in a Tunisian family with combined factor V and VIII deficiency

Congenital factor V and VIII deficiency (F5F8D; OMIM 227300) is an autosomal recessive bleeding disorder in which low levels of both FV and FVIII (usually between 5% and 30% of normal), prolonged prothrombin time (PT), and partial thromboplastin time tests (PTT) are the main clinical symptoms. Congenital factor V and VIII deficiency is mostly caused by mutations in the LMAN1 gene (70% of cases) and, more rarely, by MCFD2 gene defects (30% of cases). In the normal state, the two proteins encoded by the LMAN1 and MCFD2 genes form a cargo receptor complex involved in the transport of FV and FVIII from the endoplasmic reticulum to the Golgi apparatus. In the absence of the ERGIC-53/MCFD2 complex, the secretion of FV and FVIII is inefficient, resulting in low plasma levels and bleeding. F5F8D deficiency is extremely rare in the general population (1:1,000,000). However, its frequency increases in regions where consanguineous marriages are practiced [1]. We present in this study the identification of a novel MCFD2 gene missense mutation by direct sequencing.

Case Report

The proband is a 22 years old female, diagnosed at the age of 20. She is offspring of a consanguineous marriage. The levels of her FV and FVIII are 10% and19% respectively. Results of direct sequencing of the LMAN1 and the MCFD2 genes demonstrate that our patient had a point mutation (c.265G>A) in MCFD2 exon 3 resulting in substitution of a negatively charged hydrophilic Asp residue to a polar uncharged Asn amino acid at position 89 (p.Asp89Asn) in a homozygous state, while her mother was heterozygote (figure 1). Mutations were absent in the LMAN1 gene, only 3 polymorphisms were identified in this gene which are the Val39Arg and the GT deletion in intron 4 two of them in the heterozygous state and the Arg117Arg in the homozygous state (Table 1).

The p.Asp89Asn change occurred in a highly conserved amino acid residue among species and using PolyPhen, this mutation is predicted to be probably damaging with a score of 0.992 (sensitivity: 0.59; specificity: 0.96). The p.Asp89Asn represents the second mutation identified at the same position. The first mutation was a p.Asp89Ala and it was demonstrate that it abolishes MCFD2 binding to LMAN1 [2]. Our finding lets us able to suspect that the amino acid residue change in the same position may increase the protein unfolding rate and consequently prevent the formation of the MCFD2-LMAN1 complex with the same rate.

In Tunisia a founder effect mutation in the LMAN1 gene was identified in the Tunisian Jews of Djerba Island [3]. In the Muslims population, until now only three families with F5F8D were identified (Table 1), two of them are already described [4, 5] and the third is present in this study in whom we identified a novel MCFD2 gene missense mutation by direct sequencing.

Figure 1 : DNA sequence surrounding nucleotide 265 at MCFD2 exon 3
(A) DNA sequence of the normal control. The normal nucleotide (G) is indicated by an arrow.
(B) DNA sequence of the patient, a homozygous mutation G to A transition at nucleotide 265.
(C) DNA sequence of the patient mother, G to A transition is present in the heterozygous state.

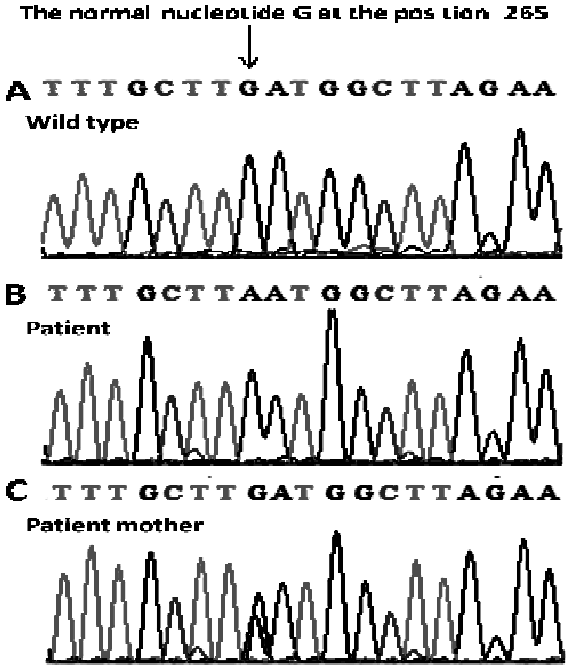

Table 1 : All reported F5F8D Tunisian patients: their geographic origins, gender, mutations and polymorphisms

Family	Origin	Patients	Gender	Mutations	Polymorphisms	References
Family 1	Tunis	3	M	p.Asp81His* in the homozygous state	None	10, 11
				p.Val100Asp* in the heterozygous state		
Family 2	Nabeul	1	F	p.Arg202X in the heterozygous state	Arg117Arg in the homozygous state	10
					GT deletion in intron 4 in the heterozygous state	
Family 3	Sousse	1	F	p.Asp89Asn* in the homozygous state	Val39Arg in the heterozygous state	This study
					Arg117Arg in the homozygous state	
					GT deletion in intron 4 in the heterozygous state	