Haemophilia

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LETTER TO THE EDITOR

Identification of novel and recurrent mutations in Tunisian haemophilia B patients

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Haemophilia B disease is a recessively inherited X-linked bleeding disorder which results from deficiency of factor IX (*F9*). Haemophilia B has a frequency of approximately 1 in 25 000 men worldwide [1]. Haemophilia B results from heterogeneous mutations spread throughout the *F9* gene [2].

According to the World Federation of Hemophilia Report on the annual global survey 2007, 51 haemophilia B in Tunisia have been reported [3]. In this first study on Tunisian haemophilia B, we report the molecular analysis of 16 unrelated haemophilia B families. Patients involved in this study were from the Hemophilia Treatment Center, Aziza Othmana hospital, Tunisia. Informed consent was obtained from all patients.

Molecular analysis was performed using the following strategy: polymerase chain reactions for the entire coding sequence of the *F9* gene were prepared as described previously [4]. The mutation detection protocol was performed by dHPLC on a WAVE DNA Fragment Analysis System (Transgenomics, San Jose, USA). Altered profiles detected by dHPLC were sequenced using ABI Dye Terminator Cycle Sequencing (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) and analysed using a capillary sequencer Genetic Analyser ABI PRISM310 (Perkin-Elmer Applied Biosystems, Foster City, CA, USA [4]. Results were analysed using BLAST (http://www.ncbi.nlm.nih.gov/blast) program against the normal *F9* gene sequence (GenBank Accession No. K02402) and the mutations were compared with the haemophilia B mutation database (http://www.umds.ac.uk/molgen).

To evaluate the nature of missense mutations, we used PolyPhen (Polymorphism Phenotyping) (*http://genetics.bwh.harvard.edu/pph*).

Our cohort is composed of 30 patients belonging to 16 unrelated families who represent 60% of total haemophilia B Tunisian population. A total of 15 different mutations were detected (Table 1), except for one family that did not show any mutations. In addition, the polymorphism g.20421A>G in exon 6 was also identified in five families. Five novel mutations were identified in five patients including 2 missense mutations, 1 nonsense mutation, 1 splice site mutation and one small deletion.

For patient Hb 2, a deletion of CAG sequence from the 17795 to 17797 position inducing the loss of the last acid Ala173 in exon 5 (The numbering of the amino acids is according to the Swiss-Prot PZES (P00740)).

For patient Hb10, a T to A substitution at nucleotide position 113 which changes a Cys acid in a codon stop (Cys27X) in exon 1, which will result in nonsense-mediated RNA decay and produce a severe phenotype as no protein will be translated, has been revealed.

Patient Hb12 shows an acceptor splice substitution at the position 10507 in intron 4 (+2T > C). For Patient Hb14, a substitution of T to A at the position 31286 in exon 8, change the Cys 435 to Ser.

Patient Hb16 present a substitution of G to A at the position 30932 in exon 8, which change Ala 317 to Thr. Replacement of a non-polar amino acid residue by a polar one is likely to affect the function, secretion or stability of the protein.

Using PolyPhen these two mutations are predicted to be probably damaging with a score of 1.000 and 0.995 respectively.

The question of whether these two candidate mutations Cys435Ser and Ala317Thr are pathogenic and alter the three-dimensional structure and function of *F9* protein needs further investigation.

However, as the latter mutations along with, Ala173Del, Ala317Thr and Cys435Ser occurred at amino acid residues highly conserved among different species, they may be involved in the *F9* destabilization.

Compared with previously published reports [5], we found that the two deletions identified in our patients bearing a severe disease. However, in our patient cohort the two nonsense mutations were associated with different phenotypes, severe and moderate disease respectively - in patient Hb17(FIX:C level of 4) which is at variance with the majority of entries on the haemophilia B database for this mutation (which cite FIX:C and antigen levels of <1 for most of the 56 examples). Our observation in Hb17 is actually the exception for this particular mutation and for most nonsense mutations in general.

In patient Hb3, we could not detect any mutations in the *F9* gene using first dHPLC (no altered profile was observed) then sequencing. It is possible that this family might have pathological translocation, duplication or inversion in the factor IX gene leading to the disease. Further investigation is needed.

To our knowledge this study is the first comprehensive molecular analysis of haemophilia B patients in Tunisia. Five novel mutations were identified and our data are globally in agreement with other reports in the international database. When requested, the data obtained from this study will be used for carrier testing and prenatal diagnosis. The identification of the mutations can also be used to estimate the risk of inhibitor development. It can also be valuable when planning future studies including gene therapy.

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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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Table 1. Mutations and polymorphism in FIX gene in Tunisian haemophilia B patients.

Family	Number of patients/family N = 30	Patient	FIX level*	Severity	Region	Mutation	Amino acid change	Comments	Polymorphism Ala194Thr	Reffered**
1	1	Hb1	<1	severe	Exon 5	g. 17743 T >C	p.Ser156Pro	Missense		3
2	3	Hb2	<1	severe	Exon 5	g.17795-17797del CAG	p.Ala173 Del	In-frame 3 codon deletion		This study
3	1	Hb3	<1	severe		Not determined				
4	1	Hb4	<1	severe	Exon 4	g.10430G>T	p.Gly106Cys	Missense	yes	1
5	3	Hb5 & Hb6	<1	severe		Deletion of all exons				
6	3	Hb7 & Hb8	<1	severe	Exon 8	g.31287G>A	p.Cys435Tyr	Missense		8
7	1	Hb9	<1	severe	Exon 8	g.31221G>A	p.Gly413Gln	Missense	yes	2
8	2	Hb10	<1	severe	Exon 1	g.113T>A	p.Cys27X	Nonsense		This study
9	1	Hb11	3.2	moderate	Exon 2	g.6364C>T	p.Arg-42Trp	Missense		51
10	4	Hb12	20	mild	Intron 4	g.10505+2T>C		Donor splice		This study
11	3	Hb13	<1	severe	Exon 2	g.6451G>T	p.Glu72X	Nonsense	yes	3
12	1	Hb14	<1	severe	Exon 8	g.31286T>A	p.Cys435Ser	Missense		This study
13	3	Hb15	<1	severe	Exon 2	g.6365G>A	p.Arg42Gln	Missense		70
14	1	Hb16	30	mild	Exon 8	g.30932G>A	p.Ala317Thr	Missense	yes	This study
15	1	Hb17	4	moderate	Exon 8	g.30863C>T	p.Arg294X	Nonsense		56
16	1	Hb18	<1	severe	Exon 6	g.20518C>T	p.Arg226Trp	Missense	yes	37

FIX, factor IX.

*FIX activity (FIX: C) was assessed using an activated partial thromboplastin time-based one-stage clotting assay using CKPrestSTAGO® and FIX deficient plasma(STAGO®) with STACOMPACT.STAGO®.

Please note that aminoacid numbering given is according to the Swiss-Prot PZES (P00740). To convert to the haemophilia B mutation database remove 46 to aminoacid numbers.

** In ''Referred column'' we indicate if the mutation is new or it is recurrent and how many times it was cited in the haemophilia B mutation database.

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