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LETTER TO THE EDITOR

Identification of Novel Mutations in Exon 14 of the *F8* Gene in Malaysian Patients with Severe Hemophilia A

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In the management of haemophilia A patients, it is important to identify the underlying mutations in order to better understand the pathogenesis and develop appropriate treatment strategies to effectively treat the disease. As yet, mutations are commonly found in introns 22 and 1 of the *F8* gene of haemophilia A patients. Intron 22 inversions and intron 1 inversions account for about 45–50% and 1–2% of severe cases respectively [1]. In the remaining severe haemophilia A patients without intron 1 or intron 22 inversions, various nonsense and missense mutations and also small or large deletions and/or insertions have been detected through previous studies [2, 3].

In this study, we focused on exon 14 of the *F8* gene as it is the largest exonic coding sequence in the gene. The large size makes it more prone to mutations compared to the other exons. Furthermore, numerous types of mutations which have been associated with haemophilia A have been found in this exon from past studies [1, 4]. Therefore, in the hopes of discovering novel mutations which have not been previously reported, this region was targeted.

As an initial pilot study, DNA was extracted from the blood samples of 11 severe haemophilia A patients from the National Blood Centre (NBC) after informed consent was obtained. Medical history and background were also obtained from all the patients. Polymerase Chain Reaction (PCR) was performed on the DNA using 16 primers to target exon 14 of the F8 gene as previously described by Zhang et al. [5]. The primers were designed to span the whole region of exon 14 in the *F8* gene and a 'GC'-clamp was added to each primer pair to promote specific binding. Agarose gel electrophoresis was then carried out on all PCR products obtained to confirm the sizes of the amplicons. PCR products which gave clear single bands upon agarose gel electrophoresis were cleaned-up using Wizard SV Gel and PCR Clean-Up System (PROMEGA) before being subjected to DNA sequencing. DNA sequencing was carried out at the Centre of Chemical Biology (CCB), Universiti Sains Malaysia (USM) using Applied Biosystems ABI 3730× 1 DNA analyzer. The sequence obtained was then compared with the FVIII gene reference sequence, NM_000132.3 in the GeneBank genetic database. The translated protein sequence was also obtained from the database with the reference sequence of NP_000123.1. All sequence changes were confirmed by forward and reverse sequencing.

Of the 11 patients that were tested, three of them (Patients 1, 5 and 10) exhibited sequence mismatches when compared to the reference sequence. A total of five different mismatches were identified including four single nucleotide substitutions and a single base deletion. The results are summarized in Table 1. All the mismatches have not been reported in the Haemophilia A Mutation Database [6] except for p.Ser1288Ser which had been reported as a single nucleotide polymorphism (SNP). Therefore, these four new mismatches could be considered novel as it has not been reported in the HAMSTeRS database. It has not been reported in the CDC Hemophilia A Mutation Project (CHAMP) mutation list as well [7]. The new mismatches found may cause severe haemophilia A by altering the structure of the final protein which may result in quantitative or qualitative changes in the FVIII protein.

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Table 1 Summary of sequence mismatches and clinical data of patients

Patient	Nucleotide change[a]	Amino acid change		Phenotype	Inhibitors	Family history
		HAMSTeRS[b]	HGVS[c]			
1	c.3078, A>C	p.Ser1007Ser	p.Ser1026Ser	Severe	Yes	No
	c.3325, C>T	p.Gln1090X	p.Gln1109X			
	c.3864, A>C	p.Ser1269Ser	p.Ser1288Ser*			
5	c.3175, del A	p.Lys1040?	p.Lys1059?	Severe	No	No
	c.3180, A>T	p.Lys1041Asn	p.Lys1060Asn			
10	c.3864, A>C	p.Ser1269Ser	p.Ser1288Ser	Severe	Yes	Yes

[a] Nucleotide numberings refer to the *FVIII* gene reference sequence, NM_000132.3 and conform to the convention that ATG is the initiation codon

[b] Haemophilia A Mutation, Search, Test and Resource Site

[c] Human Genome Variation Society

[*] Refers to single nucleotide polymorphism reported in the HAMSTeRS database? Refers to possibility of frameshift mutation

To confirm that the sequence mismatches found in this study are actual mutations which can cause severe haemophilia A in patients, further functional studies such as F8 binding assays, need to be carried out. Association between the mismatches and the clinical presentations of the patients should also be analyzed further through correlation studies to correlate the mutations with the phenotype of the disease.

In conclusion, we report four new mutations in exon 14 of the F8 gene for the first time. These initial promising observations would certainly pave the way for a large scale study which involves a bigger sample size to determine the distribution of these mutations in the general population. Furthermore, these mutations could be of extreme importance as it might provide further insight regarding the pathogenesis of Hemophilia A and greatly assist in studying the development of FVIII inhibitors.

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