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BRIEF COMMUNICATION

Factor IX mutations in Haemophilia B patients in Malaysia: a preliminary study

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Abstract

Haemophilia B is caused by coagulation defects in the factor IX gene located in Xq27.1 on the X chromosome. Identification of mutations contributing to defective factor IX may be advantageous for precise carrier and prenatal diagnosis. We studied 16 patients from 11 families, consisting of 8 patients of the Malay ethnic group, of which 6 were siblings. Factor IX mutations have not been previously reported in the Malay ethnic group. The functional region of the factor IX gene was sequenced and mutations were identified in either the exon or intronic regions in 15 of the patients. One novel mutation, 6660_6664delTTCTT was identified in siblings with moderate form of haemophilia B. Mutations identified in our patients when linked with disease severity were similar to findings in other populations. In summary, this preliminary data will be used to build a Malaysian mutation database which would facilitate genetic counseling.

Key words: factor IX**,** Hemophilia B**,** mutation**,** clotting factor

INTRODUCTION

Haemophilia B (HB) is an X-linked inherited blood clotting disorder resulting from deficiency in the blood coagulation factor IX. Deficiency of the clotting factor is caused by mutation of the Factor IX gene. The disease has been expressed in males and diagnosis is confirmed by low activity of plasma factor IX (FIX). Mutations of this gene have been curated in the Haemophilia B mutation database.[1] The different mutations identified in factor IX have been found among patients with varying severity of HB. Patients with HB accounted for about 9% of patients with hereditary bleeding disorders in Malaysia.[2] At present, there is a lack of information of mutations of this gene in Malaysian patients. This preliminary study aims to characterise mutations of the factor IX gene in HB patients from various ethnic groups in Malaysia.

MATERIALS AND METHODS

Our study was conducted on 16 patients (8 unrelated patients and 8 siblings from 4 families). Five families had only one affected male with HB. Informed consent was obtained from each family. The disease severity of the patients was determined by coagulation factor levels. Of the cases studied, 5 were classified as severe cases of HB while 11 had moderate form of HB.

Amplification and direct sequencing of the factor IX gene was carried out using previously published primers.[3,4] Purification of amplicons was carried out using QIAquick PCR Purification Kit (Qiagen, Germany). Sequencing reactions using BigDye® Terminator v3.1 cycle Sequencing kit (Applied Biosystems, USA) were run on ABI 3130 Genetic Analyzer. Exons 1-8 of factor IX gene was sequenced in all patients, and where available, in the mothers of the patients. Factor IX sequence (Genbank Acc No: K02402) was used as a reference and mutations were numbered accordingly.[5]

RESULTS AND DISCUSSION

Mutations were identified in 15 patients with an exception of 1 patient with factor IX activity levels of 5% (Table 1). Mutations were identified at the promoter, conserved exon amino acids and the donor/acceptor splice sites. One novel

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TABLE 1: Mutations found in Malaysian patients diagnosed with haemophilia B

Patient	Factor IX activity (%)	Region	Mutation	Affected amino acid	Ethnic group
F1	H8= 4.0	promoter	6T>A		Chinese
	H9=5.5	promoter	6T>A		Chinese
F5	H3=<1.0	exon 2	6365G>A	R-4Q	Malay
F6	H4=<1.0	exon 2	6396_6397delAG	frameshift	Chinese
F2	H5=1.8	exon 2	6460C>T	R29X	Malay
	H6=<1.0	exon 2	6460C>T	R29X	Malay
F7	H1= <1.0	intron 2	6490G>A		Indian
F3	H13=1.35	intron 2	6660-		Malay
	H14= 1.75	intron 2	6664delTTCTT		Malay
			6660-		
			6664delTTCTT		
F8	H15=1.78	intron 3	6707G>A		Chinese
F4	H10=<1.0	exon 5	17692 G>A	G93S	Malay
	H11=<1.0	exon 5	17692G>A	G93S	Malay
F9	H16=1.5	exon 5	17692G>A	G93S	Malay
F10	H2=<1.0	exon 6	20540C>A	A187D	Chinese
F11	H7=2.5	exon 6	20540C>A	A187D	Chinese

mutation was identified in this study. No factor IX inhibitors had been detected in the patients.

Promoter change 6T>A was identified in 2 brothers of Chinese origin with moderate severity of the disease. The males of family 1 (F1) were first diagnosed at 3 and 5 years of age. Their mother is a carrier with coagulation activity of 59.3%. Previous studies reported the mutation in 2 neonates with severe phenotype with increased levels of FIX coagulant activity on the onset of puberty as commonly seen in HB Leyden.[6,7]

Frameshift mutation 6396_6397delAG causing a non-functional factor IX was found in an affected male with severe HB, whose mother tested as a carrier. F2 siblings and their mother carried R29X mutation. They were of Malay origin. There have been no previous reports of factor IX mutations among the Malay ethnic group. The 2 mutations were expected to cause functional loss of factor IX that had contributed to greater severity HB.

Patient H2 and H7 with severe and moderate phenotype of HB respectively had 20540C>A mutation. This mutation was previously described in severe cases.[8] However, the effect of the mutation on disease severity is yet to be understood. The mother of H7 was heterozygous for the mutation.

6365G>A (R-4Q) change in the CpG dinucleotide region was found in a Malay patient with severe HB. Donor splice site mutations, 6490G>A and 6707G>A were identified in patients with severe and moderate haemophilia B respectively. This findings concurs with a number of other studies.[3,9] Novel intronic change 6660_6664delTTCTT (5bp) was found in 2 siblings of Malay origin with moderate phenotype. Mutation 6660_6663delTTCT (4bp) was previously found to cause 2% of normal factor IX activity.[8] Their mother is a carrier with heterozygous mutation.

17692G>A was found in siblings (H10 and H11) of Malay origin with severe form of HB. The patients' mother is a carrier with low levels of plasma factor IX. Patient H16 with moderate phenotype carried the same mutation in HB, which was diagnosed in 3 generations. However, the mutation could not be tested in other patients within the family. The mutation was previously described in severe and moderate cases.[3,7]

Double mutations and mutations in catalytic domain of the protein were not identified in our patients. This was probably due to the small sample size. In this study, we found that differences in disease severity could be linked with the type of mutation the patients. Information on factor IX antigen levels indicating the levels of the mutated protein present in the blood were not available. The patient in whom no mutation was detected could have had mutations in the intronic sequences and the region upstream to promoter. This preliminary study reports a part

of the factor IX mutations found in the different ethnic groups in Malaysia. Further studies on the mutation spectra of the HB cases in Malaysia would be useful.

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