Indian J Med Res 142, July 2015, pp 85-87
DOI:10.4103/0971-5916.162130

Correspondence

High heterozygosity frequency of three exonic SNPs of factor V gene (*F5*): implications for genetic diagnosis

Sir,

Isolated coagulation factor V (FV) deficiency (parahaemphilia) is a rare autosomal recessive bleeding disorder affecting both sexes with a prevalence of about one in one million[1]. Patients with FV deficiency exhibit mild to severe bleeding symptoms with clinical manifestations ranging from ecchymosis, menorrhagia, epistaxis to haemarthrosis and intracranial bleeds[1,2]. The factor V (*F5*) gene is located on chromosome 1q23, spans about 80 kb and has 25 exons and 24 introns with a 7 kb mRNA which encodes a protein of 2224 amino acids. The complete *F5* cDNA sequence has already been published[3,4]. More than 100 mutations and 700 polymorphisms have been reported in the *F5* mutation database[5].

Among all coagulation disorders, FV deficiency is the least characterized disorder at the molecular level. Patients with FV deficiency generally exhibit a bleeding tendency of variable severity; intracranial and gastrointestinal haemorrhages, haematomas and haemarthroses are also reported in a few patients. Major phenotypic determinant in FV deficiency has been reported to be platelet FV levels[6]. Though the disease is rare in countries like India where consanguineous marriages are common, there is a high prevalence of autosomal recessive disorders including FV deficiency. Except one report[7], there is no published study on the molecular characterization of FV deficient cases from India. Direct sequencing of the gene though accurate, is not cost-effective due to the large size of the gene and heterogeneity of mutations and is thus difficult to implement in many laboratories. Indirect method of gene tracking is an extremely simple and accurate method, when it is based on intragenic markers. However, assessing the informativeness of the marker in each population is important as there is a considerable variation in the heterozygotic frequency of these markers in different populations[8-10]. In the present study, polymorphisms in *F5* were analysed in a group of healthy individuals with an objective to utilize them for genetic diagnosis in FV deficient families by direct DNA sequencing.

The study was conducted at the Department of Haemostasis and Thrombosis, National Institute of Immunohaematology, Mumbai, India, including 65 unrelated healthy subjects from the Institute. After obtaining informed written consent, 10 ml blood sample was collected from each participant in EDTA and DNA was extracted from these samples using commercial kits (Invitrogen, CA, USA). PCR amplification was performed using primers designed by primers 3 and UCSC genome browser (Sigma Aldrich, India). The PCR reaction mixture consisted of 1U *Taq* polymerase (Bioron, Ludwigshafen, Germany), 1.5 mmol/l magnesium chloride, 1 μmol/l of the forward and reverse primers and 150 ng of DNA. The primer sequences and PCR conditions for exon 13 A2663G (K830R), A2684G (H837R) and exon 16 A5380G (M1736V) polymorphisms were as follows:

Exon 13 (637 bp) - forward (5'-3') TGCTGACTATGATTACCAGA, reverse (5'-3') GAGTAACAGATCACTAGGAGG.

PCR conditions: Denaturation at 94°C for 5 min followed by 35 cycles of denaturation (95°C, 40 sec), annealing (56°C, 40 sec), and extension (72°C for 40 sec) along with a final extension at 72°C for five minutes.

Exon 16 (286 bp) - forward (5'-3') GAGGCAATACAATTTACTC, reverse (5'-3') CAGTGTGATTTAATTAGGAG.

Table. Heterozygosity frequencies of polymorphic markers of *F5*

Exon	dbSNP rs-number	Nucleotide change	Amino acid change*	Allele frequency (%)			Hetero-zygotic frequency (%)	Cumulative frequency (%)
				AA	AG	GG		
13	rs4524	A2663G	K830R	18/65 (27.69)	36/65 (55.38)	11/65 (16.92)	36/65 (55.38)	67.69
13	rs4525	A2684G	H837R	18/65 (27.69)	39/65 (60)	8/65 (12.30)	39/65 (60)	
16	rs6030	A5380G	M1736V	12/65 (18.46)	33/65 (50.76)	20/65 (30.76)	33/65 (50.76)	

*Numbering is according to Ref 3. dbSNP, single nucleotide polymorphism database

PCR conditions: Denaturation at 94°C for 5 min followed by 30 cycles of denaturation (95°C, 1 min), annealing (55°C, 2 min), extension (72°C for 1 min), followed by a final extension at 72°C for seven minutes.

Amplified products were subjected to direct sequencing of the exons and the intron - exon boundaries of *F5* using an ABI 3130 genetic analyzer (Applied Biosystems, USA). The study protocol was approved by the Institutional Ethics Committee.

A total of 12 polymorphisms were detected, of which three showed high heterozygosity frequency in our population *i.e.* exon 13 A2663G (K830R), A2684G (H837R) and exon 16 A5380G (M1736V) (Table). The cumulative heterozygosity frequency of these three markers was 67.69 per cent.

During the course of the study, a family was referred from Uttar Pradesh for antenatal diagnosis in the first trimester of pregnancy. After obtaining a detailed medical and family history, blood samples of both parents and index case were collected and the chorionic villus sampling (CVS) was done at 11th weeks of pregnancy in the mother. DNA from blood samples and CVS was isolated using commercial kits (Invitrogen, USA) and subjected to gene tracking analysis by direct DNA sequencing technique using the above three markers *i.e*. exon 13 A2663G (K830R), A2684G (H837R) and exon 16 A5380G (M1736V) polymorphisms. The diagnosis could be successfully offered to this family using exon 13 A2663G (K830R) polymorphism. The family was non-informative for exon 13 A2684G (H837R) and exon 16 A5380G (M1736V) markers. As the mother was homozygous for this marker, the foetus is either normal or a carrier of the mutation. Thus the diagnosis of an 'unaffected' foetus was offered. The child was subsequently followed up after delivery and was found to be normal for factor V levels with a normal genotype.

The present study shows that these three markers can successfully be used in carrier diagnosis and prenatal diagnosis in majority of the FV deficient families in India. Due to the high cost involved in direct DNA or mRNA analysis involved in mutation detection, indirect linkage analysis using such highly informative polymorphic markers may be considered as the method of choice for genetic diagnosis in developing countries.

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