THREE NEW MUTATIONS IN THE FACTOR IX GENE

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INTRODUCTION

Hemophilia B, an X-linked recessive bleeding disorder, that occurs in about 1:30,000 males is caused by wide range of mutations in the factor IX (F9) gene. The F9 gene maps to Xq27, spanning about 34 kb. The coding sequence distributed in eight exons (*a-h*) is transcribed in to 2.8 kb mRNA. It encodes a single polypeptide chain, comprised of a pre and prosequence (27 and 19 amino acids respectively) and mature factor IX protein consisted of 415 amino acids.

The coagulation factor IX is a vitamin K-dependent serine protease and plays an important role in coagulation pathway. Depending on the residual factor IX activity, the disease is classified as severe (<1%), moderate (1-5%) or mild (6-30%), and the phenotype severity is related to the type and position of the mutation in the F9 gene. Characterization of molecular events in F9 has been area of intense research since its sequence was deduced. Detection of the mutations is important for precise genetic diagnosis as well as for studying the genotype-phenotype correlation in this disease. At present over 560 mutations are identified within F9 gene, thus establishing the remarkable allelic heterogeneity of hemophilia B (Haemophilia B mutation database; *http://www.kcl.ac.uk/ip/petergreen/haemBdatabase.html)*.

In this paper we present data on three novel mutations: Gln246Pro; Phe349Leu and -AT affecting codons 237/238, identified in two Macedonian and one Bulgarian hemophilia B patients.

MATERIAL AND METHODS

Polymerase Chain Reaction (PCR); Single Strand Conformation Polymorphism (SSCP) analysis

Genomic DNA was amplified by PCR using oligonucleotide primers and cycling conditions described by *Mahajan A., et al. in Haematologica 89(12):1498-1503, 2004.* The SSCP was performed on BioRad DeCode System (Bio-Rad Laboratories, Hercules, CA, USA). PCR products were loaded onto a non-denaturing 12% acrylamide/bisacrylamide (39:1) gels. Electrophoresis was performed at constant power of 25W, at 4°C for about 20 hours. PCR fragments were visualized with silver staining of the gel. SSCP analysis of PCR-amplified fragments from these patients identified a single PCR product with aberrant mobility relative to normal control.

DNA sequencing

To identify the nucleotide substitutions responsible for altered electrophoretic mobility, each of the PCR fragments were sequenced using Big Dye terminators v2.1 (Applied Biosystems) on an ABI Prism 310 Genetic analyzer (PE Applied Biosystems, Foster City, CA, USA).

RESULTS AND DISCUSSION

The data for the three new mutations are given in Table 1. All three new mutations are located in exon h, affecting normal catalytic function of these protease. The novel missence mutations occurred at a highly conserved region when compared with dog, mouse and bovine F9.

Gln246Pro missence mutation was found in a Macedonian patient with severe form of disease (fIX <1%). SSCP analysis of the first part of exon *h* (Exon *h*-I) in the PCR-amplified fragment from this patient revealed a distinctly abnormal pattern. Direct sequencing revealed an A→C transversion at nucleotide 30858 leading to amino acid change of glutamine to proline at codon 246 (Figure 1A). In the hemophilia B database only one change in this codon was described in a severely affected patient with C→A change at nucleotide 30857, leading to Gln246Lys amino acid change.

Phe349Leu missence mutation was found in Macedonian patient with moderate form of disease (fIX 3%). SSCP analysis of exon *h* in his family indicate that his mother was a carrier for the mutation. A novel C→A alteration found at nucleotide 31168, results in a missence Phe→Leu mutation in the catalytic domain of the factor IX protein (Figure 1B).

A deletion of two nucleotides 30832/833 or 30834/835 affecting codons 237/238 of the catalytic domain, was found in a Bulgarian hemophilia B patient and his cousin, with severe form of disease (FIX<1%). This frameshift mutation leads to a premature stop codon in exon *h*, and truncated factor IX in its catalytic domain (Figure 2).

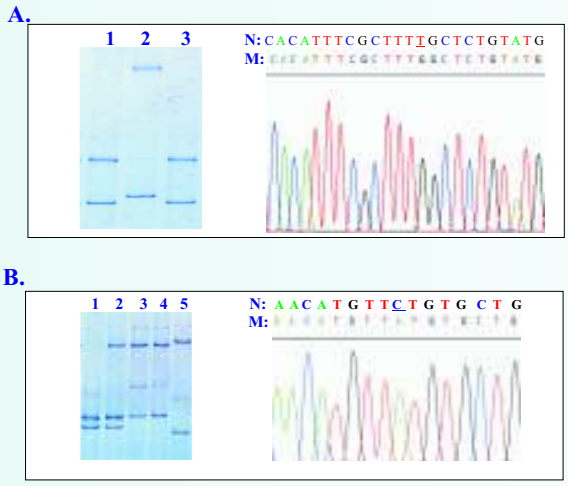

Figure 1. Representative photograph of SSCP analysis and sequencing reactions identifying mutations in hemophilia B patients from Macedonia

A) Novel missence mutation Gln246Pro in exon *h*, identified in a moderate hemophilia B patient from Macedonia (line 2); Normal control (line 1 & 3);
B) Nucleotide 31168 change C→A leading to missence mutation Glu456Val, identified in a moderate hemophilia B patient (line 1) and his mother (line 2); Nonsence mutation Arg338Stop, found in Bulgarian patient (line 5); Normal control (line 3 & 4)

Table 1. Novel F9 gene mutations

Patient	fIX:C	Severity	Nucleotide	Mutation	Conservation*
H133	<1%	severe	30858	Gln246Pro CAA→CCA	Partially generic sequence
H38	3%	moderate	31168	Phe349Leu TTC→TTA	Partially generic sequence
H91	<1%	severe	30831/32	Cd.237/238 -TA Frameshift	

*** Partially generic sequence - identical in all species of factor IX and is identical in one or two of the related proteases (factor VII; factor X and protein C**

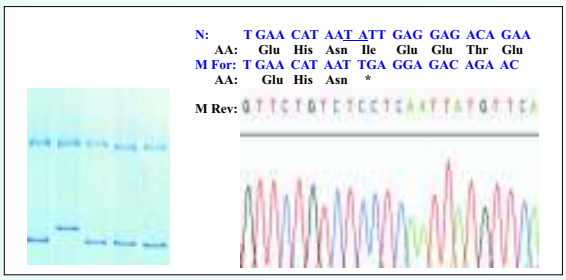

Figure 2. Representative photograph of SSCP analysis of exon h and sequencing reaction identifying deletion of two nucleotides -TA leading to frameshift mutation after codon 237 of the F9 gene

ACKNOWLEDGMENT

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