Splice junction mutations in factor IX gene resulting in severe hemophilia B

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Mutations at splice junctions of factor IX gene are rare in hemophilia B (1). Using PCR amplification followed by direct DNA sequencing, we have determined the nucleotide sequence of the factor IX gene in two patients with severe hemophilia B (FIX:C <1%, FIX:Ag <1%). The amplified fragments total 2720 bp and include the entire coding region, the 14 exon/intron junctions, from 83 bp 5′ to the transcripted sequence and extending to 90 bp 3′ to stop codon 416 of the factor IX gene (2). DNA sequences from both patients are identical to the published normal sequence, except for one mutation in each patient; a 4 bp deletion (**tgag** or **gagt**) at nucleotide numbered (6491−6494 or 6492−6495) in patient 1 and a single point mutation (**g** to **a**) at nucleotide number 30821 in patient 2. The deletion is in the consensus sequence for the donor splice junction in intron 2 of the gene. The point mutation is at the acceptor splice junction of intron 7 and exon 8. Both mutations violate the **gt/ag** rule which is nearly invariant for genes in eucaryotic organisms. Each of these mutations involve a critical nucleotide at the 5′ or 3′ end of an intron that will result in the loss of splicing function necessary for converting the precursor mRNA to the mature mRNA.

The point mutation identified in patient 2 is identical to one reported in a Japanese patient (3). Since patient 2 is an American Black, the origin of this mutation is certainly different from that of the Japanese patient. The 4 bp deletion can be detected by acrylamide gel electrophoresis of a PCR amplified fragment of 152 bp (normal) or 148 bp (patient) that includes the exon 2/intron 2 junction. The point mutation found in the second family abolishes an HphI site that can be distinguished from the normal DNA on an amplified fragment that includes the intron 7/exon 8 splice junction. Thus, both mutations can be rapidly detected by acrylamide gel electrophoresis of an amplified fragment without blotting and hybridization to isotopic-labelled probes.

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Synonymous polymorphism in the coding sequence of human 3-beta hydroxysteroid-5-ene dehydrogenase (HSD)

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Source/Description: Genomic DNA or HSD exon 3. DNA blots were probed with HSD cDNA clone 1/6 or B3 (1).

Polymorphism: BglII (AGATCT) identifies a single base pair substitution at position 1012 in the coding sequence of HSD. On genomic blots, allele 2 yields a band of 5 kb, which is not found for allele 1 (right hand panel of Fig. 1). When the PCR product of exon 3 is digested with BglII, allele 1 yields DNA fragments of 719 bp and 207 bp, allele 2 yields a fragment of 926 bp (see Fig. 1 left panel for DNA gel; center panel for blot probed with clone 1/6). Eight independent alleles were sequenced from nt 885−1099. From nt 1006, allele 1: CGAGATCTGGCG. allele 2: CGAGATTTGGCG

Chromosomal Location: 1p13.1 (2); 1p13 (3).

Mendelian Inheritance: In two families (21 individuals). cDNA probes are available from RGS.

Frequency: allele 1 = 0.61 allele 2 = 0.39 (from 28 unrelated males and females).

Other Comments: PCR amplimers were: 5′-CGTGGTTGGC-ACCTCTT-3′ and 5′-GGAGCTTGATGACATCT-3′. Taq polymerase (Promega): 30 cycles at 92.5°C 1 min, 50°C 30 sec, and 72°C for 1 min. The DNA product was precipitated and then digested with BglII. The sequencing primer was 5′-A-GCTTCCTACTCAGGCC-3′. EMBL databank accession no: X55997H.

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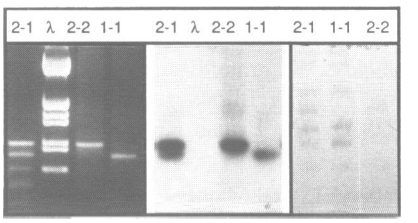

Figure 1. Left hand panel: DNA gel of amplified exon 3, center panel shows the corresponding Southern blot (the 207 bp fragment of allele 1 has run off the gel) λ = phage lambda EcoRI/HindIII markers). Right hand panel shows genomic Southern blot, 5 kb band is marked with a dot.

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