Two Novel Point Mutations Identified in the Human Coagulant Protein Factor VII Gene

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We have identified two novel point mutations in the human coagulation Factor VII (FVII) gene in two families originally from Santo Domingo. These mutations result in loss of both FVII antigen (Ag) and coagulant activity. Sequencing the promoter region (400 nucleotides) and all exons and splice junctions revealed the two mutations detailed in the table below.

Muta-tion	Zygosity	Region of gene	FVII Activity	FVII Ag	Clinical Severity and Symptomatology
-60 T C	double heterozygote with H348R	pro-moter	<1%	<3%	Mild to moderate Joint hemorrhages, mucocutaneous bleeds
H348R	homozygote	exon 8	<1%		Mild to moderate Joint hemorrhages, mucocutaneous bleeds
	heterozygote	exon 8	43%		Asymptomatic

The molecular mechanism responsible for the promoter mutation (ACTTTG ACTCTG) at -60 before the translation start site was investigated by gel mobility shift assays and transient transfections in HepG2 and HeLa cells. Gel mobility shift assays using a radio-labeled oligonucleotide probe spanning from -76 to -46 and human liver nuclear extracts revealed the loss of nuclear protein binding to a site previously shown to bind the liver-enriched transcription factor HNF-4. Transient transfections were performed in the hepatocyte-like cell line HepG2 using either 186bp of the mutant or wildtype FVII promoter sequence (-185 to +1 of the FVII gene) to drive expression of the human growth hormone reporter gene. Studies revealed the loss of promoter activity in the construct containing the -60 mutant sequence compared to the wildtype construct. Studies in HeLa cells, a cell line that does not naturally synthesize HNF-4, cotransfected with the FVII promoter constructs and an expression plasmid containing HNF-4 cDNA, revealed the loss of ability of HNF-4 to transactivate the FVII promoter. These experiments reveal FVII deficiency due to the loss of the HNF-4 binding site similar to the mutation previously described at -61. The -60 promoter mutation occurs in a region of the FVII promoter that, in the conserved ACTTTG HNF-4 binding motif of the human coagulant protein Factor IX (FIX) promoter, would be expected to result in the clinical phenotype of Hemophilia B Leyden, an antigen negative FIX deficiency that recovers post-puberty. As yet, there is no evidence of FVII recovery in these 13 year old peri-pubescent twin boys. The point mutation in exon 8 encoding the catalytic domain of FVII results in a histidine to arginine substitution in a highly conserved region in vitamin K-dependent coagulation proteins.

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