Factor IX_{Basel}: a Swiss family with severe haemophilia B having a point mutation in EGF type B domain

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Haemophilia B is an X-linked disorder caused by mutations affecting the factor IX gene. Genetic defects of the factor IX gene are heterogeneous, including gene deletions, insertions, and point mutations. We have studied a large, Swiss family with 43 members, 9 of whom are affected, spanning 3 generations. Genomic DNA from the haemophiliacs and healthy males of this family were amplified in vitro by a modified asymmetric PCR, utilising a single internal primer (1). The amplified exons were directly sequenced by the dideoxy chain terminating method, using a T7 sequencing kit (Pharmacia).

Sequence analysis revealed a single base mutation of a T→C transition at nucleotide 10418 of the factor IX gene (2). This results in a substitution of arginine for the cysteine at codon 56 in exon 4 (Figure 1), within the epithelial growth factor (EGF) type B domain of the factor IX protein. This mutation abolishes a disulphide bridge, probably with cysteine 71, and introduces a positive charge which is expected to destabilize local tertiary structure. This may alter the folding properties of the peptide backbone, thus leading to the observed clotting activity of less than 1% in this family.

The factor IX defects, previously reported in the EGF type B domain, are all associated with mild haemophilia B, including factor IX_{Alabama}, London 7, Durham, London 6, Oud en Nieuw Gastel (8%–14% of normal activity) except factor IX_{New London} (1% of normal activity) causing severe haemophilia B (3, 4, 5, 6, 7). The factor IX_{Basel} mutation is the first demonstration of the functional importance of Cys 56 in the fourth exon of human factor IX gene, resulting in severe haemophilia B.

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Figure 1. Part of the nucleotide sequence of the (5'–3') of exon 4, obtained with asymmetric PCR, and sequence analysis using exon 4 specific primers: 5' CAGTAGTTTGTCTGACCC 3' (A), 5' ATAAGGTGAGTCGGAACATC 3' (B), 5' GAGTACCAAGTTTCAAAGGG 3' (internal). The nucleotide differences between normal and FIX_{Basel} are indicated by arrows.