

## A Post-Translational Modification of Factor VII Protein Caused by a Homozygous 15bp Insertion Mutation in the Human FVII Gene

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The molecular basis for FVII (factor VII) deficiency in a 3-year-old girl from Oman has been investigated. The index case, born of a consanguineous marriage, presented with easy bruising and epistaxis. Investigations showed a factor VII coagulant activity (FVII:C) of <1% and factor VII antigen (FVII:Ag) of 10%. DNA studies identified a homozygous 15bp in-frame insertion within exon 8, at nucleotide 10685 between codons 217 and 218. This insertion-type mutation consists of a duplication of the preceding 15bp (codons 212 to 217) suggesting that it has arisen by slipped mispairing between copies of a direct repeat sequence (GCGAGCACGAC). Modeling of the crystal structure of factor VII shows that the 15bp insertion leads to the formation of an extra loop within the catalytic domain of the molecule. Such a mutation is likely to distort the folding of the protein resulting in a failure of secretion or to the rapid removal of the variant protein from the plasma. To study this in more detail, mutant FVII cDNA was prepared from the patient's DNA by overlapping PCR. Wild type FVII cDNA (FVII WT) and mutant FVII cDNA (FVII M) were expressed in COS cells and FVII:Ag measured in cell lysates and supernatants after 48hr. In cells transfected with either the WT or the mutant FVII cDNA constructs, the level of FVII:Ag in the lysates from the +mutant construct were normal. However, in the supernatant, FVII:Ag levels in the mutant construct were 5-10% of those measured in the WT supernatant. In pulse-chase experiments carried out with cells transfected with the mutant FVII cDNA and pulsed for 30 min with S35-methionine, a reduced amount of FVII protein with a higher electrophoretic mobility under non-reducing conditions was detected in the conditioned media at 60, 120, and 240 min of chase. We conclude that the 15bp insertion causes improper posttranslational modification or misfolding of the FVII protein.

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