EXPLORATION OF THE GENETIC BASIS OF COAGULATION FACTOR VII DEFICIENCY IN A MALTESE FAMILY

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Introduction

Congenital coagulation Factor VII deficiency is a rare coagulation disorder charterised by haemorrhages occurring spontaneously in the severely deficient patient or after surgical challenge in those who are mildly affected (Mariani et al., 1978). Factor VII deficiency is characteristically inherited in an autosomal recessive manner. The frequency of this disorder has been estimated to be one per 500,000 persons worldwide. Definitive diagnosis of Factor VII deficiency rests on a specific assay for Factor VII coagulant activity (functional analysis), or for Factor VII antigen level (immunochemical analysis), or both. Functional variants of Factor VII have been described based on different reactivity with tissue thromboplastins from several species (Roberts and Foster, 1987).

Factor VII deficiency in Malta was first identified in a single family in July 1991. The proband was a girl born in 1987. She had no history of bleeding tendency and Factor VII deficiency was diagnosed during pre-operative coagulation studies prior to tonsillectomy.

Studies were performed in order to explore the genetic basis of coagulation Factor VII deficiency in this Maltese kindred. Factor VII coagulant assays were performed on 11 members of the kindred. These assays were performed four times on plasma samples from the proband, siblings and parents, employing a tissue thromboplastin from a different species each time. The thromboplastins were ox, porcine, human and rabbit, brain tissue thromboplastin. Factor VII coagulant assays were performed once on plasma samples from other members of the kindred, employing rabbit brain tissue thromboplastin.

A molecular probe capable of hybridising with the Factor VII gene and suitable for mapping DNA abnormalities of the same gene was synthesised. Genomic DNA was isolated from the leukocytes of a peripheral blood sample from 16 members of the affected kindred. These genomic DNA samples were stored for future analysis utilising the synthesised probe.

Methodology

Study 1

Factor VII Bioassay

Factor VII coagulant assays were performed on plasma samples using a modified one-stage Prothrombin Time test. The modification consisted of the use of Factor VII-deficient plasma as substrate plasma, to make the test specific for Factor VII. The assays were performed on an automated coagulometer (Automated Coagulation Laboratory^{TM} 300, Instrumentation Laboratory). Ox, porcine and human brain tissue thromboplastins were prepared by acetone drying of the fresh brain, while rabbit brain tissue thromboplastin was purchased from Instrumentation Laboratory.

Study 2

DNA Studies

Genomic DNA was isolated by a technique which gently lysed the leukocytes and solubilised the DNA, followed by physical methods which purified and precipitated the DNA.

The Factor VII gene probe that was synthesised was 653 base pairs long and complementary to a region towards the middle of the Factor VII gene. This probe was synthesised by Polymerase Chain Reaction technology, which is an in vitro method for the enzymatic synthesis and amplification of specific DNA sequences, using two oligonucleotide primers that hybridise to opposite strands and flank the region of interest in the template DNA. Normal genomic DNA was used as template for the synthesis of the Factor VII gene probe.

The primer pair were selected by the use of primer design software (Primer Detective^{TM}, Clotech Laboratories Inc.) and were synthesized on a DNA synthesizer (Applied Biosystems). The Polymerase Chain Reaction was carried out in an automated thermal cycler (DNA Thermal Cycler, Perkin-Elmer Cetus).

The Polymerase Chain Reaction product was positively identified as the required probe by determination of its length. This was achieved by

electrophoresis of the product and comparison of the position of the resulting band with that of PhiX/Hae III marker fragments within the gel. The identity of the product was also confirmed by restriction endonuclease mapping, employing restriction enzymes which digest the expected probe at known sites.

Results

Study 1

Factor Bioassay

Results of the Factor VII bioassays are shown in Tables 1 and 2. The pedigree of the proband and her relatives is shown in Figure 1.

Table 1: Results of Factor VII coagulant assays with a panel of tissue thromboplastins from different species, on plasma from proband, siblings and parents. Results of Factor VII coagulant activity below that of the normal control are shown in bold print.

No	Subject	Factor VII Coagulant Activity with Various Thromboplastins (%)			
		Rabbit Brain thrombo-plastin	Human Brain Thrombo-plastin	Ox Brain thrombo-plastin	Porcine brain Thrombo-plastin
1	Proband	**12**	**3**	**2**	**2**
2	Father	**27**	**12**	**4**	**8**
3	Mother	45	46	40	40
4	Sister	46	**38**	20	**31**
5	Brother	**10**	**2**	**1**	**1**
	Normal Control	43	41	18	37

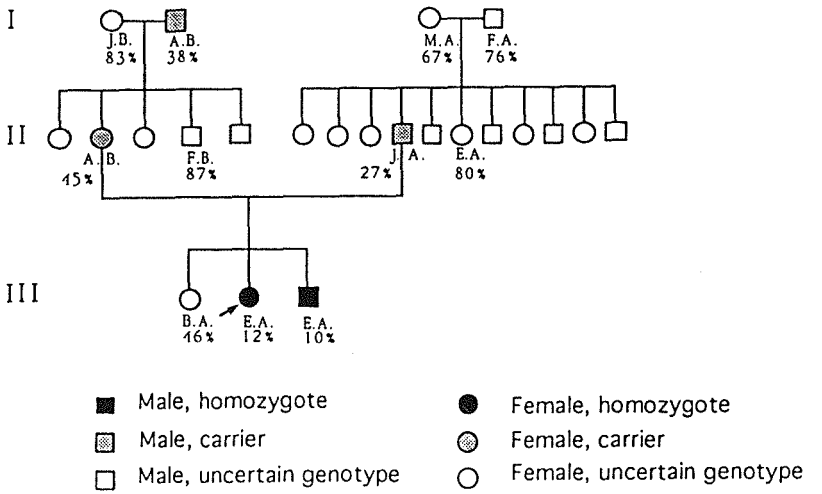

Figure 1: Pedigree of proband and her relatives. Percentages correspond to Factor VII Coagulant Activity (with rabbit brain tissue thromboplastin)

Table 2: Results of Factor VII coagulant assays with rabbit brain tissue thromboplastin, on plasma from members of the kindred (namely grandparents, aunts and uncles of proband). Result of Factor VII coagulant activity below that of the normal control is shown in bold print.

No	Subject Initials	Factor VII Coagulant activity with Rabbit Brain Thromboplastin %
6	A.B.	**38**
7	J.B.	83
8	F.B.	87
9	F.A.	76
10	M.A.	67
11	E.A.	80
	Normal Control	52

Study 2

DNA Studies

The synthesised Factor VII gene probe (653 bp) can be seen in Figure 2 as a band located slightly above the 603 bp fragment of the PhiX/Hae III marker.

Restriction endonuclease mapping confirmed the identity of the synthesised probe, since fragments of expected length were produced.

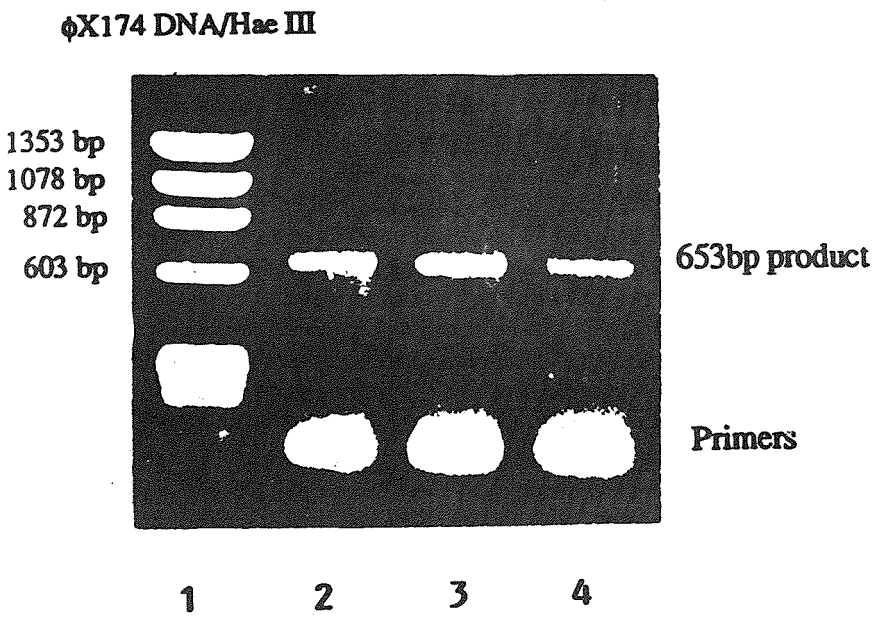

Figure 2: Agarose gel analysis of the product of the Polymerase Chain Reaction product. Lane 1 includes Hae III-digested PHiX 174 DNA as marker. Lanes 2, 3 and 4 include the product of the Polymerase Chain Reaction (the 653 bp probe).

Discussion

This study represents an initial approach to the characterisation of the functional defect and its molecular basis, causing hereditary Factor VII deficiency in this kindred.

While bioassay studies provide basis for understanding structure-function relationships in the Factor VII protein, DNA studies provide information on the molecular deficiency at the DNA level.

Such studies also form the basis for diagnosis and carrier detection of family members and provide the necessary information for counselling.

Additionally, by understanding the mechanism of the disease, future therapeutic intervention may be possible.

References

Mariani G., Mannacci P.M., Mazzucconi M.G., Capitanio A. (1978). Treatment of congenital Factor VII deficiency with a new concentrate. Thrombos. Haemostas. 39: 675-682.

Roberts H.R., Foster P.A. (1987). Inherited disorders of prothrombin conversion. In: Haemostasis and Thrombosis, 2nd ed. Editrs: Colman R.W., Hirsh J., Marder V.J., Salzman E.W. Philadelphia: J.B. Lippincott Company, 169-181.