Factor V Leiden (FV R506Q) in Families with Inherited Antithrombin Deficiency

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Summary

We investigated the presence of the gene mutation of factor V, FV (R506Q) or factor V Leiden, responsible for activated protein C resistance, in DNA samples of 127 probands and 188 relatives from 128 families with antithrombin deficiency. The factor V mutation was identified in 18 families. Nine families were available to assess the mode of inheritance and the clinical relevance of combined defects. The factor V and antithrombin genes both map to chromosome 1. Co-segregation of the defects on opposite chromosomes was observed in three families. Co-segregation with both defects on the same chromosome was demonstrated in four families. In one family a de novo mutation of the antithrombin gene and in another a crossing-over event were most likely explanations for the observed inheritance patterns.

In six families with type I or II antithrombin deficiency (reactive site pleotropic effect), 11 of the 12 individuals with both antithrombin deficiency and the factor V mutation developed thrombosis. The median age of their first thrombotic episode was 16 years (range 0-19); this was compared with a median age of onset of 26 years (range 20-49) in 15 of 30 carriers with only a defect in the antithrombin gene. One of 15 subjects with only factor V mutation experienced thrombosis at ages of 3 years. In three families with type II heparin binding site deficiency, two of six subjects with combined defects experienced thrombosis; one was homozygous for the heparin binding site defect.

Our results show that, when thrombosis occurs at a young age in antithrombin deficiency, the factor V mutation is a likely additional risk factor. Co-segregation of mutations in the antithrombin and factor V genes provides a molecular explanation for severe thrombotic episodes in related generations. The findings support that combinations of genetic factors underly differences in thrombotic risk in families with thrombophilia.

Introduction

Several studies have established resistance to activated protein C as an independent and strong risk factor for thrombosis (1-4). Activated protein C resistance is associated with the same point mutation of the FV gene, a G→A substitution at nucleotide position 1691 that predicts replacement of Arg(R) 506 by Gln(Q) (factor V Leiden) (5-7). Unlike other genetic risk factors for thrombosis, the frequency of the factor V Leiden allele is high in the general population: 2-6% of normal individuals carry the factor V mutation (5, 8, 9).

It is becoming increasingly clear that the combination of two (or more) mutant alleles of genes that encode proteins involved in the clotting mechanism determines the thrombotic risk in individuals (10). In homozygous individuals this combination consists of two mutant alleles of one and the same gene. It was recently found that the homozygous state for the factor V mutation gives a high, 80-fold, increased risk of thrombosis (11). Likewise, the presence of a second genetic risk factor might explain higher numbers or severe thrombotic events observed in some families with one other inherited risk factor. In a study of families with a combination of protein C gene mutation and the factor V mutation, 75% of the carriers of two risk factors had experienced a thrombotic episode, compared to 31% (protein C) and 13% (factor V) of related carriers of a single defect (12). Finally, several families are reported in the literature in which the combination of two (or three) independent segregating risk factors for thrombosis is associated with severe thrombotic episodes (13, 14, 15).

Inherited antithrombin deficiency arises from an uncommon autosomal disorder and is associated with an increased risk of venous thromboembolic disease. The gene encoding antithrombin has been localised to chromosome 1q23-25 (16). With the human locus for the factor V gene mapped to chromosome 1q21-25, only an estimated distance exists between 3 and 11 cM from the antithrombin gene (derived from the NIH/CEPH Collaborative Mapping Group linkage map and a recent study) (5, 17, 18). Antithrombin deficiency may be classified in two major types, based upon the results of functional and immunological assays. Type I deficiency is characterised by reduced functional and immunological antithrombin levels, both approximately 50% of normal. Type II deficiency results from the presence of a functionally inactive protein, usually with approximately normal antigen levels. Type II deficiency may be subclassified into variants with reactive site (type II RS), heparin binding site (type II HBS) and pleiotropic effect (type II PE) defects (19). This classification is clinically important. The type II HBS variants are associated with only a mildly increased thrombotic risk, except when present in homozygous state (20). Excluding this subtype (type II HBS), about 50% (range 15 to 100%) of individuals with antithrombin deficiency are symptomatic in reported families (21).

Because of the high allele frequency of the mutated factor V gene, it is likely that many members of families with antithrombin deficiency also carry this allele. We therefore investigated DNA samples of a large...
group of kindreds, registered with antithrombin deficiency in our centres, for the presence of this combination. Detailed data of nine families were available to investigate the effect of the chromosomal location on the segregation pattern and to assess the clinical relevance of the combination of risk factors.

**Patients and Methods**

*Patients and families.* DNA of 127 probands and 188 relatives was available from 128 unrelated families with inherited antithrombin deficiency. 111 families (110 probands) presented with antithrombin type I or type II RS/PE deficiency and 17 families (17 probands) with antithrombin type II HBS deficiency. Diagnosis of inherited antithrombin deficiency was initially based on anti-thrombin activity levels of less than 80 %, and in the majority the genetic defect has been identified (see reference 22). Since each of the centres is a specialized reference centre for diagnosis and treatment of familial thrombophilia, most of the families registered were referred because of unexplained, recurrent thrombosis occurring at young age. Notably, the probands and family members had been diagnosed over a period of time before the identification of the factor V mutation. The therefore unbiased information of the occurrence of venous thrombosis was obtained by routine medical history, and included self-reported thrombophlebitis, deep venous thrombosis and pulmonary embolism.

**Detection of the factor V Leiden mutation and mutations in the antithrombin gene.** For all 315 DNA samples a 220 bp fragment of exon 10 (intron 10 of the factor V gene was amplified, followed by digestion with the restriction enzyme Mnl I, as described previously (5, 12). The 220 bp fragment of a normal factor V gene is cleaved by Mnl I resulting in fragments of 37, 67, and 116 bp. When the factor V mutation is present, one Mnl I site is lost resulting in a 67 and 153 bp fragment. These fragments were visualised following electrophoresis on a 2 % agarose gel. The antithrombin gene mutations were investigated by PCR and genomic sequencing of affected exons, as described (23, 24).

**Results**

Screening for the factor V Leiden mutation was performed in DNA samples of 127 probands and 188 related family members. The factor V mutation was found to be present in 18 families. In 15 of these families the proband carried the factor V mutation in combination with a defect in the antithrombin gene. In three families the factor V mutation was identified in relatives of the proband.

Detailed medical histories with emphasis on manifestations of thrombosis were available for 47 individuals from nine families with both the factor V mutation and an antithrombin defect. These nine
families comprised six families with type I or type II RS or PE deficiencies (35 subjects) and three families with type II HBS deficiency (12 subjects). Eight of these 47 subjects carried an antithrombin mutation, five the factor V mutation, 18 the combination, and 16 subjects had no defects.

The inheritance patterns of the factor V mutation and antithrombin deficiency are shown in Fig. 1. In pedigree one, two and presumably the defects segregated independently, apparently due to their presence on opposite chromosomes. Consequently, family members with both defects were observed only in one generation. In pedigree four, five and six, the factor V mutation and the mutant antithrombin gene segregated over at least two generations. Similarly, in pedigree five two daughters appear to have inherited a chromosome from their father that carries both defects.

Pedigrees eight and nine show complicated patterns of segregation. In pedigree eight, a de novo antithrombin mutation has occurred in pedigree eight individual II.1 (19). It will become certain whether or not the new defect is linked to the factor V Leiden allele when family members in the next generation are investigated. In pedigree nine, II.1 inherited the two defects on opposite homologous chromosomes. Of the daughters, III.1 inherited the chromosome carrying the factor V mutation and III.2 the chromosome with the antithrombin gene defect. In contrast, III.3 inherited a chromosome that carries the factor V mutation as well as the antithrombin gene defect, an A insertion in codon 169. The simplest explanation for this inheritance pattern assumes a crossing over event at meiosis in the father, resulting in recombination onto one chromosome.

In Table 1 the underlying defects and available clinical details are described for the nine families with combined defects. To assess the clinical relevance of the combined defects, we compared the histories of thrombosis in the six families, with the type I or II (RS or PE) deficiencies, of the histories from 30 subjects of nine other families. These nine families were selected on the basis of the availability of clinical data.

Table 1: Details of individuals from nine families with factor V Leiden and antithrombin deficiency

<table>
<thead>
<tr>
<th>Pedigree number</th>
<th>Sex</th>
<th>Antithrombin deficiency</th>
<th>Factor V Leiden*</th>
<th>Natural history</th>
<th>Reference</th>
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<tr>
<td>1</td>
<td>M</td>
<td>Arg202Glu</td>
<td>GG</td>
<td>recurrent stroke</td>
<td>(23)</td>
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<tr>
<td>2</td>
<td>F</td>
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<tr>
<td>6</td>
<td>M</td>
<td>MII.1</td>
<td>NT</td>
<td>cerebral vascular accident</td>
<td>(23)</td>
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<tr>
<td>7</td>
<td>M</td>
<td>MII.2</td>
<td>NT</td>
<td>myocardial infarction</td>
<td>(23)</td>
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<tr>
<td>8</td>
<td>M</td>
<td>MII.3</td>
<td>NT</td>
<td>no stroke</td>
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<td>9</td>
<td>M</td>
<td>MII.4</td>
<td>NT</td>
<td>recurrent stroke</td>
<td>(23)</td>
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**Bold pedigrees are carriers of both defects.** * = Packaging, ** = Deep vein thrombosis, $ = Pulmonary embolism, $ = No, no plasma and DNA available, **CVA = Cerebral vascular accident, $SMI = Myocardial infarction.

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Individuals who are heterozygous for the heparin binding variant are not reported to have an increased risk of developing venous thrombosis (26). The numbers involved in the families with this deficiency subtype are too small to allow any statistical estimate of the risk for thrombosis due to the presence of the factor V mutation. However, the factor V mutation was present in both symptomatic family members and it seems a potential additional risk factor in this deficiency subtype. Still, four of the six carriers of both defects were asymptomatic. This suggests either a moderate risk for these family members, constituting of the risk of the heparin binding defect itself and the factor V mutation, or the presence of additional risk factors in the two symptomatic members. In support of this, the exceptional young age (14 months) and severity of thrombosis in one family member is explained by the presence of such a third risk factor, the homozygosity for the heparin binding defect.

Our findings clearly support the contention that the presence of simultaneous genetic defects increased the risk for thrombosis in anti-thrombin type I and II RS or PE. 92% of carriers of two risk factors had experienced a thrombotic episode, compared to 54% of carriers of a single antithrombin gene defect. Our observed one of five symptomatic individuals with only factor V mutation is in accordance with an extended family study in which approximately 25% of the relatives with APC resistance were reported to have experienced thrombosis (27). Very striking was the early median age of onset for the carriers of both defects, being 16 years (range 0-19). By comparison, in the families with antithrombin type I or type II (RS, PE) deficiency, the median age was 26 years (range 20-49) for symptomatic family members. The recognition that combinations of distinct genetic risk factors might be responsible for severe thrombosis is relatively recent. While the combination of genetic risk factors has been described, it is worth noting that the present study shows a combination of defects that alter the function of both the heparan sulphate-antithrombin and the protein C/protein S/factor V pathways. It appears from the results presented here that simultaneous defects in these pathways result in severe manifestations of thrombotic disease.

The increased risk for thrombosis caused by the presence of two risk factors resulted in a familial tendency for thrombosis and therefore referral and study of these families in our centres. It is for further study to unravel whether additional genetic and/or environmental risk factors determine the risk of thrombosis in the families without the factor V mutation, which were referred to our centres. In conclusion, when the factor V Leiden mutation segregates in antithrombin deficient families, thrombosis may occur at a very young age in individuals with both genetic defects. If factor V gene mutation is detected in families with antithrombin deficiency, family investigation should be performed to determine whether both defects segregate on one chromosome because of a strongly increased risk for family members in the next generation.

Acknowledgements

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References


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