

## A Novel Genetic Risk Factor for Venous Thrombosis

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**Featured Article:** Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996; 88:3698–703.<sup>2</sup>

Since the early 1980s our group had been interested in the molecular basis of familial thrombophilia. We studied families with a history of thrombosis to learn about the association between venous thrombosis and abnormal plasma phenotypes. Through these studies, hereditary deficiencies of the anticoagulant proteins antithrombin, protein C, and protein S were discovered. These rare disorders were caused by a large variety of loss-of-function mutations in the coding genes. The first gain-of-function mutation associated with venous thrombosis [the G1691A substitution in the *F5*<sup>3</sup> gene, coagulation factor V (proaccelerin, labile factor)] was also found by studying an abnormal plasma phenotype (resistance to activated protein C). This mutation, which predicts the replacement of Arg506 by Gln (factor V Leiden) in one of the cleavage sites for activated protein C, is found in about 3% of the white population (1).

In the report cited above, we described the discovery of a second prothrombotic gain-of-function mutation [the G20210A substitution in the *F2* gene, coagulation factor II (thrombin)]. This time we had used a different approach and started with the hypothesis that mutations in the *F2* gene were associated with venous thrombosis. Bert Poort sequenced all of the exons and the 5' and 3' untranslated regions (UTRs) of the prothrombin gene in 28 selected thrombosis patients and 5 healthy individuals. Apart from the previously reported polymorphisms, he found novel sequence variation at 1 site in the 3' UTR of the gene, which was present in 5 of the patients and none of the volunteers.

This G20210A variation was in the very last position of the 3' UTR. We were initially rather skeptical of the relevance of this finding; however, when we subsequently found that only 1 of 100 healthy individuals carried this variant, we became more enthusiastic and continued with the genotyping analysis for this mutation in a large case-control study of venous thrombosis (Leiden Thrombophilia Study). The results convincingly demonstrated that heterozygous carriers of 20210A have a 2.8-fold increased risk of a first venous thrombosis. Furthermore, we demonstrated that the 20210A allele was associated with 30% higher plasma prothrombin concentrations and that a high plasma prothrombin concentration itself was associated with an increased thrombosis risk. The G20210A mutation was one of the first gain-of-function mutations in a 3' UTR that was shown to be relevant to disease (2). It is extremely rare in non-Caucasians and is slightly more prevalent in southern Europe than in northern Europe. The age of the mutation has been estimated to be about 24 000 years. Its presently wide distribution in Caucasians suggests an evolutionary advantage for this mutation.

Numerous studies have now confirmed the significance of the *F2* G20210A variant (rs1799963) as a risk factor for a first venous thrombosis, and thrombophilia testing often includes screening for this mutation, although the effect of this mutation on the risk of recurrent thrombosis seems modest (3). Its relatively high population frequency (1%–3%) and its well-established association with venous thrombosis has stimulated its use in studying the role of blood coagulation in such common diseases as cardio- and cerebrovascular disease and placenta-mediated pregnancy complications. Recent data from prospective and case-control studies indicate that carriers of 20210A have a slightly increased risk of ischemic heart disease (relative risk, 1.17) (4).

In vitro functional studies were required to prove the causality of the G20210A change and to elucidate its mechanism. The use of various reporter constructs unequivocally showed that the G20210A change itself was the functional mutation (2, 5). Most investigators also agreed that the higher protein concentrations were caused by a more efficient processing of the 20210A-containing pre-mRNA. Opinions still diverge, however, on the reason why processing is altered by the G20210A change, which produces a CA dinucleotide.

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<sup>3</sup> Human genes: *F5*, coagulation factor V (proaccelerin, labile factor); *F2*, coagulation factor II (thrombin).

The presence of a CA dinucleotide at the 3' end of the last exon of many genes suggests that CA is the preferred substrate for poly(A) polymerase; however, most in vitro studies have observed similar distributions of poly(A) attachment sites in 20210A- and 20210G-containing pre-mRNAs (5). Processing of the prothrombin pre-mRNA is also stimulated by an upstream sequence element located in the 3' UTR of the mRNA. This element enables processing at the 20210 position, which is otherwise embedded in a region with several relatively weak consensus signals for pre-mRNA cleavage and polyadenylation (6). Thus, it is fairly easy to "improve" on the wild-type sequence as a substrate for poly(A) polymerase.

The question of how increased plasma prothrombin concentrations influence the risk of venous thrombosis is still a subject of debate. Such changes increase thrombin generation in vitro, probably by changing the ratio between the procoagulant prothrombin and the anticoagulant proteins antithrombin and protein S. Furthermore, they probably increase activated protein C resistance, owing to the inhibitory effects of prothrombin on the inactivation of factor Va by activated protein C (7).

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*acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

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