both PCR products of a patient double heterozygote for FII and FV are digested, fragments of 272, 249, 153, and 116 bp are detected (Fig 1, lanes 9 and 11).

In conclusion, the presented multiplex amplification on 2 µL of whole blood followed by a combined restriction digest of the obtained PCR products offers a very rapid, feasible, and cost-saving method for large-scale FII and FV genotype analysis.

Encarnación Gómez
Sonja C.P.A.M. van der Poel
Joop H. Jansen
Bert A. van der Reijden
Bob Löwenberg
Department of Hematology
University Hospital Dijkzigt
Institute of Hematology
Erasmus University
Rotterdam, The Netherlands

REFERENCES


The Prothrombin 20210 A Allele Is Frequently Coinherited in Young Carriers of the Factor V Arg 506 to Gln Mutation With Venous Thrombophilia

To the Editor:

Since the discovery of the factor (F) V Arg 506 to Gln mutation (FV:R506Q) as the most common inherited disorder associated to venous thrombophilia1-6 and its apparent cosegregation with other well-established inherited prothrombotic risk factors,7-12 evidence is accumulating that the association of double or multiple hemostatic defects greatly increase the penetrance of thrombotic disease. This finding raises the question whether the novel sequence variation in the prothrombin gene (20210 G to A variant),13 which has been identified as a common but probably mild risk factor for venous thromboembolism (VTE),13-16 may also cosegregate with the FV:R506Q mutation and contribute to the thrombotic tendency in subjects being affected by activated protein C (APC)-resistance.

Therefore, we read with interest the recent report by Alhenc-Gelas et al17 about the rare association between the prothrombin 20210 A allele and FV:Q506 in thrombophilic families. These investigators looked for an association of the two risk alleles in 288 subjects belonging to 26 families; 151 carried the FV:R506Q mutation and 66 had thromboses. However, no probands or family members had the 20210 A allele. Thus they concluded that the prothrombin variant does not frequently contribute to thrombosis in individuals with the FV:R506Q mutation. The question is this: Are the findings reported by Alhenc-Gelas et al17 affected by the high percentage of asymptomatic subjects studied or by the selection of patients, respectively? Furthermore, because no separated and detailed data about age or clinical settings were given for the FV:Q506 carriers, their results are difficult to assess.

We report here different and intriguing data showing a highly prevalent coinheritance of the prothrombin variant 20210 GA as an additional prothrombotic risk allele among young symptomatic FV:Q506 carriers.

After obtaining informed consent, FH genotyping was performed in 200 apparently healthy controls and unpreferentially in 200 carriers of FV:Q506, including 150 unrelated patients who had had an objectively confirmed VTE before 45 years of age. The FV genotype at nucleotide 1691 was determined by polymerase chain reaction (PCR) and Mbol I restriction analysis of PCR-amplified genomic FV DNA fragments.2,3 Screening of the prothrombin variant due to a G to A transition at nucleotide 20210 of the FH gene was performed by HindIII cleavage of a 345-bp fragment amplified by PCR using a mutagenic primer as described previously.13 The 20210 A allele was found in 4 of 200 healthy subjects with a normal FV genotype (100 men and 100 women; age range, 18 to 47 years; median age, 26 years), corresponding to a prevalence of 2%, whereas among 50 asymptomatic heterozygous FV-Q506 carriers (22 men and 28 women; median age, 31 years; range, 24 to 64 years), the prothrombin variant was detected in 2 subjects (4%). Among 115 symptomatic subjects affected by the heterozygous FV:R506Q mutation (69 women and 46 men; median age at onset of VTE, 28 years; range, 18 to 45 years), 14 (12.2%) also had the FII 20210 A allele. In the presence of the 20210 A allele, the relative risk of juvenile VTE was additionally threefold increased in patients carrying the FV:R506Q mutation in a heterozygous form (95% confidence interval, 0.8 to 11.7), which itself was found to increase the risk of VTE approximately fourfold.18 Patients affected by double heterozygous defects presented with thrombosis at a slightly younger age (median age at onset of VTE, 27 years) as compared with patients suffering from either FII 20210 A (33 years) or FV:Q506 in a heterozygous (29 years) form. In the group of 35 symptomatic patients affected by homozygous FV:R506Q mutation (21 women and 14 men; median age at onset of VTE, 27 years; range, 18 to 33 years), a coexistence of the prothrombin 20210 AG genotype was detected in 5 subjects, corresponding to a prevalence of 14%.

Persons homozygous for the 20210 A allele were not found.

With respect to the coexistence of the prothrombin variant 20210 GA in carriers of the FV:R506Q mutation, the rate observed in the presented study of relatively young thrombophilic patients was clearly higher compared with the rare association published for other populations.14-17,19 However, assuming the theory that a high proportion of combined inherited hemostatic abnormalities predispose for thrombophilia already at a young age, the significance of the uncommon coinheritance of both FV:Q506 and prothrombin variant observed in previous studies is difficult to assess; either the age was not mentioned at all17 or the majority of patients investigated were over the age of 60 years,15 much older than our patient population.14,16 By contrast, Poort et al13 reported that the prothrombin variant was identified in 18% of selected patients, segregated in 40% with the FV:R506Q mutation.13 Furthermore, the 20210 A allele possibly has a similar distinctive racial and/or geographical distribution, as has been described for the FV mutant.20 These
observations need to be kept in mind for prediction of the risk of VTE emanating in different populations from either FV:R506Q or FII 20210 GA or their coinheritance.

In summary, the high frequency of additional carriageship for FII 20210 GA found in young thrombophilic patients with the FV:R506 mutation indicates that the prothrombin 20210 A allele is an important additional risk factor for VTE and might contribute to the thromboembolic manifestations. A careful search for the prothrombin 20210 G to A variant should therefore be included in thrombophilia screening programs, particularly in young patients carrying other genetic defects predisposing for thrombosis. However, whether the coinheritance of FV:Q506 and FII 20210 GA is also associated with a higher recurrence rate of thrombotic events is one issue in an ongoing prospective study.

S. Ehrenforth
G. Ludwig
S. Klinke
M. Krause
I. Scharrer
Department of Internal Medicine
University Hospital
Frankfurt, Germany
U. Nowak-Göttl
Department of Pediatrics
University Hospital
Münster, Germany

REFERENCES


The A20210 Allele of the Prothrombin Gene Is Frequently Associated With the Factor V Arg 506 to Gln Mutation But Not With Protein S Deficiency in Thrombophilic Families

To the Editor:

A genetic variation in the 3′-untranslated region of the prothrombin gene (G to A transition at position 20210) has recently been linked to increased plasma prothrombin levels and an enhanced risk of venous thrombosis.1 The A20210 allele is present in 5.0% to 7.3% of venous thrombosis patients and in 1.2% to 2.8% of healthy controls.14 Familial thrombophilia has been shown to be a complex genetic disorder often caused by the segregation of two or more gene defects.15 Thus, individuals with combined genetic defects are at higher risk of thrombosis than those with single gene defects. However, Alhenc-Gelas et al16 did not find any patients with the A20210 allele of the prothrombin gene among 288 French subjects belonging to 26 thrombophilic families with activated protein C (APC)-resistance, suggesting a lack of gene-gene interaction between the prothrombin and factor V gene defects. To further investigate this particular gene-gene interaction, we have studied the A20210 allele of the prothrombin gene in Swedish thrombophilic families with APC-resistance and protein S deficiency.
The Prothrombin 20210 A Allele Is Frequently Coinherited in Young Carriers of the Factor V Arg 506 to Gln Mutation With Venous Thrombophilia

S. Ehrenforth, G. Ludwig, S. Klinke, M. Krause, I. Scharrer and U. Nowak-Göttl