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Venous and arterial thrombosis : associations and risk factors

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Risk of cardiovascular disease in double heterozygous carriers and homozygous carriers of factor V Leiden and prothrombin G20210A; a retrospective family cohort study

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F5 R506Q (Factor V Leiden) and *F2* G20210A (prothrombin G20210A) are risk factors for venous thrombosis.[1] The results of studies as to whether these mutations also increase the risk of cardiovascular disease (CVD) are inconsistent. A meta-analysis of 191 studies (N>150.000) calculated a 30% increased risk of CVD among single heterozygous *F5* R506Q or *F2* G20210A carriers compared to non-carriers.[2] Despite its large size, this study included too few double heterozygous and homozygous carriers to estimate the risk of CVD in individuals with these genetic traits. Therefore, we performed a post hoc analysis in a retrospective family cohort that contained a fairly large number of relatives who were double heterozygous or homozygous for *F5* R506Q and *F2* G20210A (n=52).

Details of our study have been published previously.[3-5] Briefly, 1641 first-degree relatives, aged 15 years or older, of consecutive patients (probands) with documented venous thrombosis or CVD before the age of 50 years and *F2* G20210A, high FVIII or hyperhomocysteinaemia, were enrolled after informed consent was obtained. Information on CVD and exposure to classical cardiovascular risk factors was collected by using a standardized questionnaire and reviewing medical records. All patients were screened for *F5* R506Q and *F2* G20210A. Information was obtained without knowledge of the genetic status.

Observation years were defined as the years from the age of 15 until the date of inclusion or until the date of the first thrombotic event. Incidences and 95% confidence intervals (95% CIs) were calculated under the Poisson distribution assumption. Relative risks and 95% CIs of cardiovascular disease were calculated in the double heterozygous/homozygous group, using single heterozygous carriers as a reference group. As we studied a thrombophilic cohort, we only compared single heterozygous *F5* R506Q or *F2* G20210A carriers to double heterozygous and homozygous carriers. The *a-priori* CVD risk for other relatives was deemed too high for them to be included as a reference group. To avoid bias, we excluded probands from the analysis.

To prevent the risk of CVD being based on relatives with cardiovascular risk factors, we intended to repeat the analysis after excluding all relatives with overweight/obesity, smoking, hypertension, dyslipidemia or diabetes mellitus. Unfortunately, as all relatives had at least one of these risk factors at the time of CVD, we were unable to do this. For similar reasons, another analysis was performed after excluding all relatives with concomitant thrombophilias.

The pedigrees of 500 probands (373 patients with objectively documented venous thrombosis, 107 patients with CVD, and 20 patients with both venous thrombosis and CVD) disclosed 1641 first-degree relatives aged 15 years or older. Of these relatives, 45 were not evaluable because of missing laboratory data and 1149 were found to be non-carriers of *F5* R506Q or *F2* G20210A (8% of whom had a cardiovascular event). The remaining 447 relatives were analyzed: 175 were single heterozygous for *F5* R506Q , 220 were single heterozygous for *F2* G20210A, 37 were double heterozygous for *F5* R506Q and *F2* G20210A, 8 were homozygous for *F5* R506Q and 7 were homozygous for *F2* G20210A. The clinical characteristics are summarized in Table 1. Males and females were distributed equally. The median age at inclusion was 47 years (range, 15-91). Concomitant thrombophilias were found in approximately 60% of all relatives.

During the observation period a total of 33 arterial thrombotic events occurred: 7% in single heterozygous *F5* R506Q or *F2* G20210A carriers and 12% in double heterozygous and homozygous carriers. The median age at onset was similar in both groups (53 years; range, 26-78). The annual incidence of CVD was 0.23% (95% CI, 0.13-0.79) in single heterozygous *F5* R506Q or *F2* G20210A carriers and 0.36% (95% CI, 0.13-0.79) in double heterozygous and homozygous carriers; relative risk 1.6 (95% CI 0.7-3.9) (Table 2). After exclusion of relatives with concomitant thrombophilias, the relative risk of CVD increased to 5.1 (95% CI, 1.3-22.9) in double heterozygous or homozygous *F5* R506Q and *F2* G20210A carriers compared to single *F5* R506Q or *F2* G20210A carriers.

In our study, double heterozygous and homozygous *F5* R506Q and *F2* G20210A carriers had a 1.6 fold (95% CI, 0.7-3.9) increased risk of CVD compared to single *F5* R506Q or *F2* G20210A carriers. These results did not reach statistical significance, however, they further the findings of two previous studies that found a modest association between single heterozygous *F5* R506Q or *F2* G20210A and CVD [2;6], but included too few subjects to calculate, as we did, a risk in homozygous and double heterzogygous carriers.

It is unknown why, in *F5* R506Q and *F2* G20210A carriers, the risk of CVD appears to be more than 10 times weaker than the risk of venous thrombosis. A plausible explanation is that atherosclerosis plays a major role in CVD and a smaller role in venous thrombosis.[7] Atherosclerosis is known to be associated with increased endothelial damage (of which hyperhomocysteinaemia is a marker),

procoagulant changes (such as high levels of FVIII)[8] and natural anticoagulant deficiencies.[9] This could explain the observed increased relative risk of 5.1 (95% CI, 1.2-22.9) found after exclusion of relatives with concomitant thrombophilias. However, as we did not investigate whether atherosclerosis was actually less common in these relatives (e.g with intima media thickness), these results should be treated with caution.

Some aspects of our study warrant comment. A strength of our study is that, due to the large cohort of thrombophilic families, it was possible to estimate the effect of double heterozygosity or homozygosity for *F5* R506Q and *F2* G20210A on CVD for the first time. A weakness is the retrospective design and that, despite its fairly large size, the number of double heterozygous or homozygous *F5* R506Q and *F2* G20210A carriers was too low to provide risk estimates with narrow confidence intervals. The number was also too low to make a subdivision of arterial thrombotic event types for additional sensitivity analyses. Another potential limitation of our study is referral bias, as this study was performed in 3 university hospitals. However, as we tested consecutive patients (probands), we probably reduced such a bias.

In conclusion, it is likely that double heterozygous or homozygous carriers of *F5* R506Q and *F2* G20210A have a higher risk of CVD than single *F5* R506Q or *F2* G20210A carriers. However, due to small numbers, further research is required to verify our findings.

Table 1. Clinical characterist

	Heterozygous FVL-Ptmut or Homozygous FVL or Ptmut	Single heterozygous FVL or Ptmut carriers	Total
Number	52 (12)	395 (88)	447
Male	21 (40)	185 (47)	206 (46)
Age at enrollment (years)	51 (16-85)	47 (15-91)	47 (15-91)
Arterial thrombosis	6 (12)	27 (7)	33
Age at onset	54 (41-78)	53 (26-78)	53 (26-78)
Classification			
Myocardial infarction	2 (4)	12 (3)	14 (3)
Ischemic stroke	1 (2)	8 (2)	9 (1)
Transient ischemic attack	2 (4)	5 (1)	7 (1)
Peripheral arterial thrombotic event	1 (2)	2 (0.5)	3 (2)
Relatives with no other thrombophilic defects*			
	21 (40)	153 (39)	174(39)

* No antIthrombin, protein C or protein S deficiency, hyperhomocysteinemia or high factor VIII levels.
FVL denotes factor V Leiden; Ptmut, prothrombin mutation.
Continous variables denoted as median (range), categorical variables as number (%).

Table 2. Risk of cardiovascular disease in relatives of probands with a thrombophilic defect

	Observation years	Relatives with event	Annual incidence % (95% CI)	Crude relative risk (95% CI)
All evaluable relatives				
Single heterozygous *F5* R506Q or *F2* G20210A carriers	11854	27	0.23 (0.15-0.33)	Reference
Double heterozygous or homozygous *F5* R506Q or *F2* G20210A	1646	6	0.36 (0.13-0.79)	1.60 (0.66-3.88)
Relatives with concomitant thrombophilias excluded				
Single heterozygous *F5* R506Q or *F2* G20210A carriers	4199	4	0.10 (0.03-0.24)	Reference
Double heterozygous or homozygous *F5* R506Q or *F2* G20210A	614	3	0.49 (0.10-1.43)	5.13 (1.15-2.92)

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