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The Prevalence of the Prothrombin 20210 G → A Mutation Is not Increased in Angiographically Confirmed Coronary Artery Disease

Dear Sir,

An increased risk for venous thrombosis has been established for carriers of the factor V Leiden mutation (1691 G→A) and, less frequently found, for other defects of blood coagulation inhibitor pathways (1). Initially the factor V Leiden mutation seemed not to be related to arterial diseases such as myocardial infarction and stroke, as was shown in the large cohort of the Physicians' Health Study (2). In our study in 1995 we investigated 317 patients of our heart center with angiographically diagnosed coronary artery disease (CAD) and found the same high prevalence of the factor V Leiden mutation (9.1%) as in a healthy control population of our area (9.5%) (3). However, recently it has been shown that the factor V Leiden mutation contributes to a 2.5 fold increased risk for myocardial infarction in young women (4).

Almost two years ago another procoagulant mutation, located in the 3'-untranslated region of the prothrombin gene (20210 G→A) associated with an elevated level of plasma prothrombin, was described as a moderate risk factor for venous thrombosis (5). Pathobiochemical considerations suggest that an increased basal thrombin generation resulting from the elevated level of its proenzyme may stimulate the growth of the smooth vascular muscle cells, promoting or accelerating the development of coronary atherosclerosis (6).

DNA samples from 284 patients with coronary artery disease, initially isolated for our factor V Leiden study (3), were still available. Therefore we had the opportunity to screen these DNA samples for the presence of the mutation 20210 G→A of the prothrombin gene using PCR followed by restriction analysis, originally described by Poort et al. (5). Our aim was to investigate whether the occurrence of the prothrombin mutation is more frequent in patients with coronary artery disease definitely diagnosed by coronary angiography than in a control group. Furthermore both the issue of whether or not the mutation promotes myocardial infarction by supporting intracoronary thrombus formation on pre-existing atherosclerosis and the determination of the prevalence of the mutation in patients with venous thrombosis should be addressed. For the latter goal DNA samples of 294 patients with one severe or recurrent thrombotic events were available, since for this group the clinicians had requested a factor V Leiden analysis. The venous thrombosis patients were 56% female with a mean age of 48 years (range: 17-77 years) and 44% male with a mean age of 55 years (range: 13-80 years).

Among 340 healthy controls we found 7 persons (2.0%) carrying the prothrombin 20210 G→A mutation. 6 out of 284 patients with coronary

Table 1 Prevalence of the prothrombin mutation 20210 G→A and the factor V Leiden mutation in patients with coronary artery disease (CAD), patients with venous thrombosis (VT) and healthy controls

	Prothrombin mutation 20210 G→A				Factor V Leiden mutation			
	Total N	Mut. pos.	%	Odds ratio	Total N	Mut. pos.	%	Odds ratio
CAD patients	284	6	2.1	1.03	317	29	9.1	0.95
VT patients	294	17	5.8	2.90 (1.25-6.9)*	294	121	41.0	6.70 (5.3-9.4)*
Controls	340	7	2.0	-	190	18	9.5	-

* 95% confidence interval

artery disease (2.1%) carried the mutation (odds ratio: 1.03), whereas 17 out of 294 patients with venous thrombosis (5.8%) were positive for this mutation (odds ratio: 2.90; 95% confidence interval: 1.25-6.9, Table 1). The data for the factor V Leiden mutation also revealed no increased association with CAD (odds ratio: 0.95) but it confirmed the known risk for venous thrombosis (odds ratio: 6.70; 5.3-9.4, Table 1).

124 out of 288 patients negative for the factor V Leiden mutation and 10 out of 29 patients positive for this mutation had a history of at least one myocardial infarction (odds ratio: 0.7; 0.3-1.5). 120 out of 278 CAD patients without the prothrombin gene mutation and 2 out of 6 patients carrying the mutation had a myocardial infarction (odds ratio: 0.66; 0.12-3.6). According to these data the hypothesis that the prothrombin 20210 G→A variation is a risk factor for coronary artery disease or the occurrence of myocardial infarction is not supported. So far, these results agree with the data of Ferraresi et al. (7), who found 2 persons carrying the prothrombin mutation within a small group of 90 CAD patients in comparison with 7 out of 176 control subjects.

Recently, Rosendaal and coworkers (8) reported on a group of 79 young women with myocardial infarction, aged 18 to 44 years. They found a prevalence of 5.1% for the prothrombin mutation compared with 1.6% in 381 female controls (odds ratio: 4.0). However, these particular patients are quite different to our group of CAD patients with regard to the mean age of 58 years (range 28-85 years) and the percentage of females (only 15%).

Therefore, in the case of the atypical rare myocardial infarction in young women, the prothrombin 20210 G→A mutation combined with the factor V Leiden mutation and/or in association with other risk factors, seem to be important and should be analyzed in these patients. But beyond this the present data do not indicate that mutations which will surely lead to an increased basal thrombin generation play an important role in the development of common coronary artery disease or myocardial infarction.

Correspondence to: Dr. W. Prohaska, Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany – Tel. +495731971191; FAX Number +49 57 31 97 23 07

W. Prohaska, M. Schmidt, H. Mannebach, U. Gleichmann, K. Kleesiek
From the Herz- und Diabeteszentrum NRW, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin und Kardiologische Klinik, Bad Oeynhausen, Germany

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Simultaneous G enotyping for Factor V Leiden and Prothrombin G 20210A Variant by a Multiplex PCR-SSCP Assay on Whole Blood

Dear Sir,

The understanding of the genetic basis for hereditary thrombophilia has been substantially expanded by the detection of common mutations in the genes for factor V (FV Leiden – G1691A) and prothrombin (G20210A mutation) associated with a remarkable increase in risk to develop thrombosis (1, 2). Therefore, genotyping for both factors has been included into routine diagnostic procedures. A variety of methods based on polymerase chain reaction (PCR) amplification of the corresponding gene fragments have been developed to detect either of these mutations separately. However, performing two different tests for large numbers of samples is time-consuming and cost-benefit calculations are critical, especially when considering prophylactic testing of individuals at risk. Thus, combining both mutations in a single genotyping procedure is of great practical impact. Two such approaches based on multiplex PCR and restriction analysis have been reported (3, 4). One of these procedures was further simplified by applying whole blood directly to the PCR reaction (4). Recently, two further assay procedures based on multiplex PCR and heteroduplex analysis (5) or on allele-specific multiplex PCR amplification (6) using isolated DNA have been described. Both test procedures allow the concomitant detection of a third possible risk factor for venous thrombosis, the methylenetetrahydrofolate reductase (MTHFR) C677T mutation.

We have developed an alternative assay for the simultaneous detection of factor V (FV) Leiden and prothrombin (PT) 20210 G/A dimorphism which can be also performed on whole blood. First, both gene fragments are amplified by multiplex PCR followed by testing for the type of alleles present by single-strand conformation polymorphism (SSCP). Primers used for the FV gene were essentially as described by Bertina et al. (1) with a slight modification in the reverse primer

(forward: 5'-GATGCCCAGTGCTTAACAAGACCA-3', reverse: 5'-TGTTATC-ACACTGGTGCTAA-3'). The primers for the PT gene fragment were: 5'-GGATGGGAAATATGGCTTCTA-3' (forward – nt20032-20052) and 5'-GAATAGCA-CTGGAGCATTGA-5' (reverse – nt20235-20215). Sizes of the amplification products are 268 bp for the FV gene fragment and 204 bp for the PT fragment. For multiplex PCR, 20 pmol of each of the four primers were included into the 50 µl reaction mixture containing 10 mmol/l Tris (pH 8.7), 40 mmol/l KCl, 3.5 mmol/l MgCl₂, 17 mmol/l (NH₄)₂SO₄, 0.2 mmol/l of each deoxynucleotide triphosphate (dNTP) and 1.25 units of Taq polymerase. After denaturing at 96° C for 10 min, 35 cycles containing the following steps were performed: 1 min at 55° C, 3 min at 72° C, 45 sec at 97° C. After final extension at 72° C for 10 min samples were cooled to 4° C. For genotype analysis by SSCP an aliquot of the PCR sample was mixed with an equal amount of formamide, heated to 95° C for

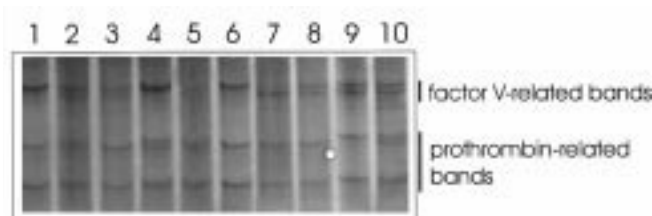


Fig. 1 Simultaneous detection of factor V Leiden mutation (G1691A) and the prothrombin G20210A variant by multiplex PCR and single-strand conformation polymorphism (SSCP) in blood samples. SSCP gel demonstrating band patterns for different genotypes. Lanes 1 and 6 – homozygotes for the two normal alleles (1691G and 20210G); lanes 2, 5 and 10 – samples heterozygous for both factor V Leiden and prothrombin 20210A variant; lanes 3 and 8 – samples heterozygous for factor V Leiden and homozygous for the normal prothrombin allele; lane 4 – homozygous for the normal 1691G allele and heterozygous for the 20210A allele; lane 7 – sample homozygous for factor V Leiden and the normal 20210G allele; lane 9 – sample from a patient heterozygous for factor V Leiden and homozygous for the prothrombin 20210A variant

Correspondence to: Prof. Dr. M. Meyer, FH Jena, FB Medizintechnik, Tatzendpromenade 1b, D-07745 Jena, Germany – Tel.: +49 3641 643127; FAX Number: +49 3641 643460; E-mail: Michael.Meyer@mt.fh-jena.de