Review

The Prothrombin G20210A Mutation - A Common Cause of Thrombophilia?

Die Prothrombin G20210A-Mutation - Häufige Ursache thrombophiler Diathesen?

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Summary: Human prothrombin is a vitamin K-dependently synthesized 72 kDa glycoprotein. The 21 kb sequence of the prothrombin gene has been mapped in the chromosomal region 11p11-q12. In 1996 a single nucleotide change (G to A transition) at position 20210 in the sequence of the 3'-untranslated region has been identified as a risk factor for venous thrombosis. Coinheritance with deficiency of protein S, protein C, antithrombin, or the coagulation factor V G1691A mutation led to a further increase of the thrombotic risk. Moreover, the G20210A mutation, if in combination with other established risk factors (e. g. smoking, metabolic parameters), was found more frequently in myocardial infarction patients than in healthy controls. An association with arterial cerebrovascular thrombosis in young patients has been shown in one study. Geographical and ethnical differences in carrier frequencies of the prothrombin G20210A mutation appeared between healthy populations, with a trend towards lower frequencies from south to north of Europe (range approximately from 0 to 4%). Screening for presence of the mutation should be performed at least in cases of otherwise unexplained thrombotic events in adults, as well as in all thrombotic events during childhood, adolescence or young adults. However, therapeutic strategies for the treatment of thrombosis in carriers of the prothrombin G20210A mutation have not been derived from clinical studies. Meanwhile, treatment should be performed similar for thrombosis patients carrying the coagulation factor V G1691A mutation or other hereditary prothrombotic risk factors.

Keywords: Prothrombin/genetics, Thrombosis/genetics; Risk Factors.

Zusammenfassung: Prothrombin wird Vitamin K-abhängig in der Leber synthetisiert und besitzt ein Molekulargewicht von 72 kDa. Die Gensequenz für Prothrombin umfaßt 21 kB und befindet sich auf Chromosom 11 (11p11-q12). Im Jahre 1996 konnte eine Punktmutation (G/A) in Position 20210 der 3'-nicht codierenden Sequenz des Prothrombin-Gens als Risikofaktor für venöse Thrombosen identifiziert werden. Das gemeinsame Vorliegen dieser Mutation mit einem weiteren prothrombotischen Risikofaktor (Protein S-, Protein C- oder Antithrombinmangel, Faktor V G1691A-Mutation) führt zu einem weiteren Anstieg des Thromboserisikos. Heterozygote Träger der G20210A-Mutation haben ein erhöhtes Risiko für Myokardinfarkte, sofern weitere, etablierte Risiken (Rauchen, metabolische Risikofaktoren) vorhanden sind. Ein häufigeres Auftreten von arteriellen Schlaganfällen bei jungen Trägern der Prothrombin-Variante konnte bislang nur in einer Studie gezeigt werden. Der prozentuale Anteil der Mutationsträger an der gesunden Bevölkerung variiert etwa zwischen 0 und 4%; innerhalb Europas ist eine Zunahme von Nord nach Süd zu erkennen. Ein generelles Screening auf Vorliegen der Mutation ist derzeit weder zu empfehlen, noch abzulehnen. In Fällen thrombotischer Ereignisse ohne erkennbare Ursache beim Erwachsenen, sowie in jedem Falle bei Gefäßverschlüssen im Kindes-, Jugend- und jungen Erwachsenenalter sollte die Prothrombin-Variante im Rahmen der Thrombophiliediagnostik bestimmt werden. Bisher wurden keine Studien zur Behandlung thrombotischer Ereignisse bei Trägern der Prothrombin G20210A-Mutation durchgeführt. Vorläufig sollte analog zur Therapie thrombotischer Ereignisse aufgrund anderer hereditärer Risikofaktoren (Protein S-, Protein C- oder Antithrombinmangel, Faktor V G1691A-Mutation) behandelt werden.

Schlüsselwörter: Prothrombin/Genetik; Thrombose/Genetik; Risikofaktoren.

Human PT is a single-chain 72 kDa glycoprotein and is vitamin K-dependently synthesized in the liver [1, 2]. Its primary structure contains 579 amino acids [3]. The mature PT molecule is composed of four domains: a Gla domain (residues 1-40), the kringle 1 domain (residues 41-155), the kringle 2 domain (residues 156-271) and a serine protease domain precursor (residues 272-579) [1, 2].

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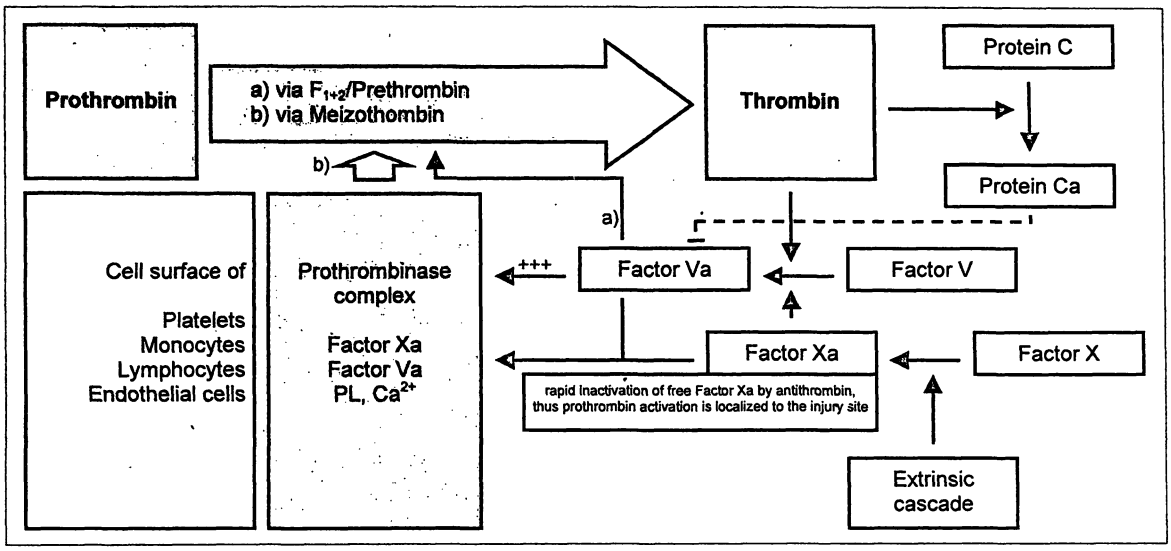

Figure 1 Role of prothrombin and its regulation mechanisms in hemostasis

PT as the inactive precursor of thrombin is a central molecule in hemostasis. After activation by coagulation factor Xa in the presence of coagulation factor Va, calcium ions, and phospholipids, which are assembled as a prothrombinase complex on phospholipid surfaces, PT is converted into thrombin. The latter is induced by hydrolysis of two internal peptide bonds (Arg_{271}-Thr and Arg_{320}-Ile), resulting in the release of 39 kDa thrombin from the carboxyl-terminal portion of PT, whereas the Gla and the kringle containing regions from the amino-terminal end of PT remain attached to the phospholipid. Although coagulation factor Va increases the conversion rate of this pathway dramatically, a mechanism acting slowly and solely with factor Xa is probably important for the initial reaction: after conversion of coagulation factor X to Xa, coagulation factor V is proteolyzed by factor Xa to factor Va. In addition, factor Xa converts PT to thrombin, which itself activates coagulation factor V. Once factors Va and Xa are available, the prothrombinase complex can assemble on cell membranes.

The serine protease thrombin has procoagulant, anticoagulant and antifibrinolytic functions. It participates in the final stage of blood coagulation mainly by converting fibrinogen to fibrin, forming an insoluble fibrin clot. Additional functions of thrombin are the activation of coagulation factors V, VIII, XIII and protein C, as well as stimulation of platelets, monocytes, lymphocytes, and endothelial cells [1, 2, 4-6].

Non standard abbreviations: CI, confidence interval; DVT, deep venous thrombosis; FV, coagulation factor V; HIND III, restriction endonuclease from *haemophilus influenzae*; MI, myocardial infarction; OR, odds ratio; PCR, polymerase chain reaction; PT, prothrombin.

PT is found in plasma concentrations of 100-200 μg/ml and has a plasma half-life of approximately 72-96 hours [7, 8] (Figure 1).

The prothrombin gene and the G20210A mutation

The complete 21 kb sequence of the prothrombin gene contains 14 exons (25-315 bp), 13 introns (90% of the gene, 84-9447 bp), a 5'-untranslated region, and a 3'-untranslated region [3, 9]. The gene has been mapped on chromosome 11 in the chromosomal region 11p11-q12 [10].

There are few mutations in the PT gene leading to hypo- or dysprothrombinemia and consequently to bleeding disorders [11]. However, due to the central role in the hemostatic system the gene encoding for PT might serve as a candidate gene not only for bleeding disorders but also for hereditary thrombophilia. Hence, in 1996 *Poort* et al. identified a single nucleotide change (G to A transition) at position 20210 in the sequence of the 3'-untranslated region as a risk factor for venous thrombosis [12].

The mechanism leading to an increased thrombotic risk in carriers of this mutation remains to be clarified. As localized in the 3'-untranslated region of the gene, there are no structural changes of the protein due to this mutation. On the one hand elevated levels of PT found in individuals carrying the A allele may constitute a risk factor by increasing the amount of thrombin generation and leading to coagulation activation. Thus, high levels of PT may be explained by higher translation efficiency or higher stability of the transcribed mRNA [12]. However, up to now experimental results

failed to confirm this hypothesis [13]. On the other hand, it therefore may also be suggested that the PT mutation is linked with a further sequence variant influencing gene expression [12].

Genotyping methods

Due to inter- and intraindividual variance of plasma PT concentrations, PT levels itself do not allow a conclusion to be drawn on the presence or absence of the mutation. Therefore, the PT mutation has to be determined by DNA analysis [14].

Poort et al. detected the mutation by direct sequencing after PCR amplification of the PT gene, in patients and families suffering from thrombophilia. A method for the detection of the PT mutation by PCR-mediated site-directed mutagenesis has been described in the same work. During amplification of a 345-bp fragment from exon 14 and the 3'-untranslated region a novel HIND III restriction site is introduced in the PCR product [12]. Because only the PCR product of the mutant allele is cut by applying the described method, no internal control for the digestion of HIND III is available for the amplified product of the wild-type allele. Hence, the method was improved by generating an internal positive HIND III digestion control for both alleles by introducing a new restriction site [15, 16].

A simple method for the rapid detection of the PT mutation using allele specific PCR has also been described. This method is especially useful for screening a large number of individuals, e. g. in population-based studies. In the first step, all individuals can be screened for presence of the A allele. Additionally, in subjects found to be positive for the A allele, a second step must be performed to distinguish between homozygous and heterozygous carriers of this allele by screening for the absence or presence of the G allele [17] (Figure 2).

Both the FV G1691A mutation and the PT variant are risk factors for venous thrombosis [12, 18, 19]. Therefore, these parameters may be taken into consideration for laboratory screening of patients suffering from thrombotic events. Methods for simultaneous one-step determination of both genotypes are now available: a multiplex PCR-mediated site-directed mutagenesis creating a Taq I cleavage site in both PCR products [20] and a method for simultaneous screening for both mutations by multiplex PCR on whole blood [21]. More recently, multiplex PCR methods for the simultaneous determination of the FV mutation, the PT mutation and the methylenetetrahydrofolate reductase C677T genotype have been described [22, 23].

In almost all studies the PCR-mediated site-directed mutagenesis method followed by HIND III restriction has been applied, according to *Poort* et al. (original or slightly modified) [12], or allele specific PCR [24-26]. In addition, denaturing gradient gel electrophoresis

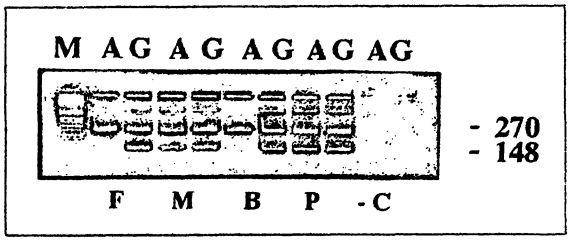

Figure 2 Gel electrophoresis of allele specific PCR products. Upper row: lane A, amplification product of the A allele; lane G amplification product of the G allele; M, marker. Lower row: F, father; M, mother; B, brother; P, DVT patient; - C, negative control. The patient, his mother and brother are heterozygous carriers of the PT mutation

[26, 27], single strand conformation polymorphism [28], or direct sequencing [29] methods were described.

The PT mutation in healthy populations, geographical and ethnical differences

The worldwide geographic distribution of the PT mutation has recently been summarized. The prevalence varied approximately from 0.7 to 4.0% with an average prevalence of 2.0%. Within Europe, there was a trend towards lower carrier frequencies from south to north: in the centers south of 50° N (Paris, Vienna, Ferrara, Tel Aviv), the carrier frequency was 3.0% (95%-CI 2.3-3.7%), whereas in centers located north of 50° N (Malmö, Manchester, Sheffield, Amsterdam, Leiden) the carrier frequency was 1.7% (95%-CI 1.3-2.2%) [30]. The PT mutation was rare in the Icelandic population (0.9%) [31], and in Greek Cypriots the carrier frequency was 4.0% [26].

In North America prevalences ranged from 1.6 to 2.4%, whereas in South America (Brazil) a prevalence of 0.7% was found [24,29,32,33]. The mutation could not be detected in populations from Africa and Asia, with the exception of African Brazilians (2.0%) [26,32-35] (Table 1 and Figure 3).

The G20210A mutation and the risk of venous thrombosis

The PT mutation is clearly associated with an increased risk for venous thrombosis. Since the first report by *Poort* et al. [12], there have been several publications confirming the initial findings: most investigators included only patients suffering DVT [12, 28, 31, 36] but sometimes DVT was summarized with pulmonal embolism [26, 29, 32, 37-40] or other thrombotic manifestations [16]. Moreover, this mutation has been demonstrated to be a risk factor for cerebral-vein thrombosis, especially for women using oral contraceptives [41]. Carrier frequencies in venous thrombosis collec-

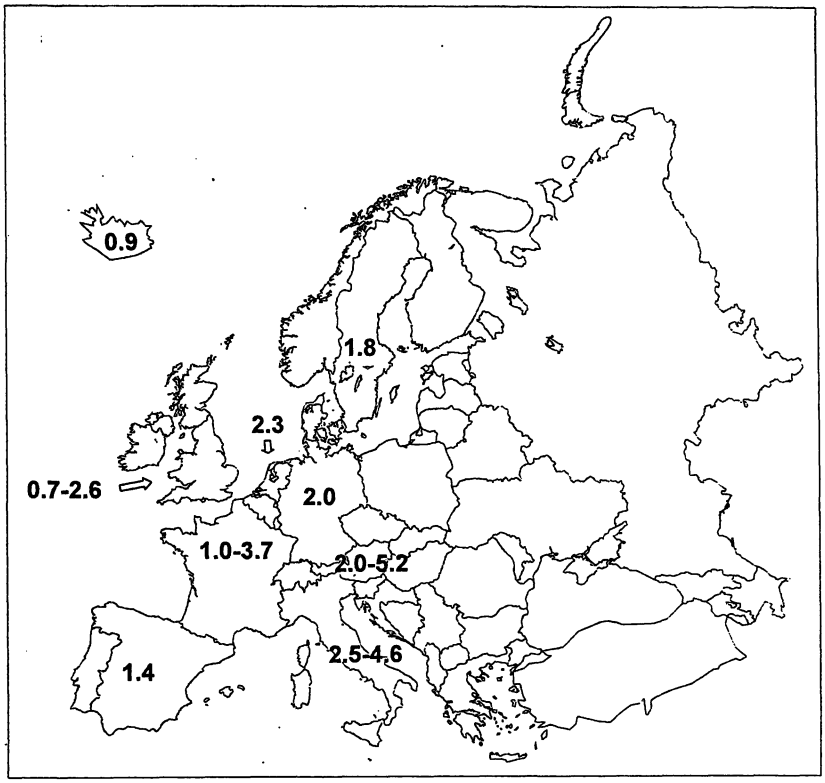

Figure 3 Prevalence of the PT mutation in non-thrombotic populations of European origin (% of carriers). For details see Table 1

tives ranged from 4.0 to 20%, and in healthy controls or newborns from 0 to 4%, resulting in ORs from 2.0 to 11.5. However, looking at DVT patients from Iceland, one study provides a contrary result: although the FV G1691A mutation constitutes a risk factor for venous thrombosis in these subjects as in other European populations, the PT mutation was found only in 1 out of 99 patients and 1 out of 108 healthy controls [31].

The age of patients ranged between 3 and 89 years, mostly without definition of subgroups for gender and age. *Poort* et al. found an increased risk of thrombosis in PT mutation carriers of all age groups in a Dutch collective (all patients, 6.2%; controls, 2.3%), whereas Hillarp et al. found a higher prevalence of the mutation only in those Swedish thrombosis patients older than 60 years of age (11% carriers; controls, 5%) [12, 36]. However, these contradicting results may be due to a selection bias (young patients were underrepresented in the Swedish study) or to further risk factors, which may add to the thrombotic risk depending on the geographic area [42].

The thrombotic risk among homozygous sickle cell disease patients in West Africa is not affected by the PT mutation: 120 patients were tested negative for the mutation, indicating that it is not common in West Africa [34]. As for arterial thrombosis, presence of the PT mutation in antiphospholipid syndrome patients did not play a major role in the occurrence of thrombosis [43, 44] (Table 2).

The G20210A mutation and the risk of arterial thrombosis

Two major manifestations of arterial thrombosis have been investigated: stroke [24, 26, 28, 29, 32, 47-51] and MI [25, 26, 28, 29, 32, 52, 53]. In some studies patients suffering coronary heart disease, stroke, peripheral artery disease and other manifestations were summarized [26, 29, 32, 35]. The number of patients investigated varied from at least 20 to at most 220, whereas the controls consisted of 20-400 healthy volunteers, patients without history of thrombosis, or newborns, 0.7-5.2% of whom were carriers of the PT mutation.

The PT mutation in patients with cerebrovascular disease

The overall prevalence of the PT mutation in cerebral ischemia patients varied from 0 to 10% [24, 26, 28, 29, 32, 47, 48]. However, up to now, only one study has proven the PT mutation to be a significant risk fac-

tor for cerebral ischemia. In this study, highly selected stroke patients aged less than 50 years were investigated (Table 3). Despite the lack of statistical significance, some of the other investigations revealed interesting results. A study on young male and female stroke patients (aged 18-49 years) showed a trend towards a higher prevalence of the mutation in the patient group compared with the controls if a known (possible) cause of stroke was present (protein S or protein C deficiency, arterial dissection, cardiac embolism). This result may suggest that the PT mutation alone is not a strong risk factor for cerebral ischemia but may precipitate arterial thrombosis in patients with thrombophilia or additional risk factors for stroke [47]. This assumption was supported by the results of a study performed on both older and younger cerebral ischemia patients of both sexes. No different prevalences of the mutation in older and younger subjects were found, but in four out of six stroke patients carrying the PT mutation a cause of the thrombotic events

Table 1 Geographic distribution of the PT mutation. Based on recent publications, abstracts not considered. In healthy subjects no homozygous carriers of the PT mutation were found.

Region		n	Population	Ages (years)	Carriers (%)
America					
Brazil [32, 52]	Sao Paulo, Campinas	295	newborns	-	0.7
USA [33]	Atlanta	278	healthy subjects	adult	2.5
USA [24, 25]	West Washington state	382	general population (females)	18-44	1.6
USA [29]	New York state[1]	50	healthy subjects, patients without history of thrombosis	22-79	2.0
Asia					
Japan [35]		77	general population	23-64	0
India [26]		41	not given	not given	0
Amazonian Indians [32]	North-East of Asia, probably related to oriental populations	83	collected randomly in two villages	14-70	0
Africa					
Somalia [26]		40	not given	not given	0
West Africa [34]		120	sickle cell disease patients	not given	0
African Americans [33]		52	not given	not given	0
African Brazilians [32]	Bahia (North-East of Brazil), population of African descent	144	students, laboratory staff, physicians	18-60	2.0
Europe					
Iceland [31]		108	healthy population	not given	0.9
Sweden [36]	Malmö[1]	282	healthy subjects	34-86	1.8
United Kingdom [61]	Sheffield	150	blood donors, healthy subjects	18-78	0.7
United Kingdom [38]	Manchester and Wigan[1]	164	blood donors	18-64	1.2
United Kingdom [37]	South-East of England	508	blood donors	not given	2.6
United Kingdom [23]	South Wales	300	general population	not given	1.3
Netherlands [12]	Leiden[1]	474	general population	16-73	2.3
Germany [60]	Frankfurt	200	healthy subjects	18-47	2.0
France [40]	Brest[1]	400	bone marrow donors	20-55	1.0
France [16]	Paris	30	healthy subjects	38±13	2.8
France [62]	Paris[1]	400	healthy subjects	not given	2.8
France [47]	Paris[1]	134	healthy subjects	18-45	3.7
Austria [53]	Vienna[1]	102	newborns	-	2.0
Austria [49]	Vienna[1]	96	no manifestation of thrombosis	28-91	5.2
Italy [41]		120	no manifestation of thrombosis	18-64	2.5
Italy [51]	Middle and Southern Italy	198	thrombosis free individuals	16-81	2.5
Italy [39]	South of Italy	431	healthy subjects	10-75	4.6
Italy [26]		106	no manifestation of thrombosis	adult	4.0
Italy [48]	Milan[1]	155	healthy subjects	adult	3.2
Spain [28]	Mediterranean area	291	no history of vascular disease	adult	1.4
Greek Cypriots [26]		80	not given	not given	4.0

Age, range or mean ± SD.

[1]Study center, population origin not exactly defined.

could be identified. However, the assumed causes of stroke were not stated in the study [48].

In a well defined collective of female stroke patients younger than 45 years of age, 2 out of 106 (1.9%) stroke patients were carriers of the PT mutation. 104 of them suffered arterial stroke, whereas 2 had a venous stroke. One of the two PT mutation carriers was found in the venous stroke group, the other in the arterial stroke group [24].

The PT mutation in coronary heart disease patients

There are two recent studies showing a clear association between prevalence of the mutation and the risk of MI [25, 52].

In one of them, focussing on female MI patients younger than 45 years of age, the mutation was found only in patients in whom additional established risk factors for MI were present (obesity, diabetes mellitus, hypertension, hypercholesterolemia). All patients carrying the PT mutation were smokers (carriers in patients and controls: 5.1% vs. 1.6%, OR 4.0, 95%-CI 1.1-15.1).

In the second study revealing a significant result (carriers in patients and controls: 3% vs. 0.7%, Fisher's exact test: p=0.03), performed on patients of both sexes, there was a trend towards a higher prevalence of the mutation in individuals suffering MI before the age of 45 years compared with older patients. Moreover, in all patients found to be heterozygous for the A allele, at least one further risk factor was present: current smoker, diabetes, hypertension, hyperlipidemia and, additionally, one patient was heterozygous for the FV G1691A mutation. In women, the number of carriers was higher in patients suffering MI beyond 45 years of age, whereas in male patients, the A allele was only found in patients of less than 45 years of age [52].

However, most investigators failed to identify the PT mutation as a (potential) risk factor for arterial thrombosis. This could be attributed mainly to the fact that either the subgroups were too small or that studies were performed without differentiation between localization of the thrombotic manifestation. Furthermore, a problem may arise because all investigations were retrospective case-control studies. The possibility that carriers of prothrombotic mutations may develop more severe thrombosis and therefore die prematurely more frequently than non-carriers might bias the results ob-

Table 2 The PT mutation in venous thrombosis

Study center	Diagnosis	n (male/female)	Age (years)	Carriers (%)	p^1	OR/95%-CI
USA [29]	DVT, PE	12/9	50	19[2]	0.025	11.5/1.2-110.5
Brazil [32]	DVT, PE	42/74	31 (median)/14-58	4.3	0.021	6.6/1.12-49[3]
Iceland [31]	DVT	99 (total)	not given	1.0	ns	
Sweden [36]	DVT	41/58	64/21-89[4]	7.1	0.010	4.2/1.3-13.6[3]
Netherlands [12]	DVT	202/269	47/16-70	6.2		2.8/1.4-5.6
United Kingdom [38]	DVT	219 (total)	43 (median)/17-88	5.5		4.7/1.04-21.3[3]
United Kingdom [37]	DVT, PE	177/326	DVT, 47 (median)/9-89 PE, 43 (median)/16-82	5.0		2.0/1.0-4.0[3,5]
France [40]	DVT, PE	171/195	65/15-92	4.6		4.8/1.5-19.8[3]
France [16]	patients with thrombosis	28/28	45/38±18	4.0	ns	
Italy [26]	DVT, PE	132 (total)	46/14-77	15	0.007	
Italy [41]	DVT CVT	DVT, 18/62 CVT, 9/31	DVT, 30 (median)/13-62 CVT 31 (median)/15-64	DVT, 18 CVT, 20		10.2/2.3-31.5[3] 10.2/2.3-31.5[3,6]
Italy [39]	DVT, PE	122/120	male, 39 (median)/16-81 female, 37 (median)/16-82	15[7]	<0.001[8]	3.6/2.0-6.4[3]
Spain [28]	DVT	44/38	61/22-87	7.3	0.05	
Netherlands [46]	CVT	-/1	21	heterozygous		case report
Italy [45]	Budd-Chiari syndrome	1/-	37	heterozygous		case report
United Kingdom [44]	patients with antiphospholipid syndrome	10/64	43	1.3	-	
France [43]	patients with antiphospholipid syndrome	13/32	41/25-67	2.2	ns	

CVT, cerebral sinus venous thrombosis; PE, pulmonal embolism. Age, mean or median and range or ± SD (not available in all cases). OR for comparison with a control group.

[1]p-value, comparison of carrier frequencies in patients and controls ($\chi 2$, [25, 29, 36, 61]; Fisher's exact test, [26]; two sample proportion test, [24]; not given, [32, 33, 38, 56])

[2] incl. 1 homozygous patient

[3]incl. patients with additional hereditary risk factors (protein C, protein S, or antithrombin deficiency, FV G1691A mutation)

[4]all carriers of the PT mutation were older than 60 years of age

[5]results for the entire patient collective

[6]all female patients carrying the PT mutation were users of oral contraceptives

[7]incl. 4 homozygous patients

[8]for allele frequencies

Table 3 The PT mutation in cerebrovascular disease and in arterial thrombosis of different localization

Study center	Diagnosis	n (male/female)	Age (years)	Carriers (%)	p^1	OR/95%-CI
USA [24]	stroke	-/105	37/18-44	1.9		1.2/0.1-6.9
France [47]	stroke	72/53	41/18-49	6.4	ns	
Austria [49]	stroke	10/10	39±8	10	ns	
Austria [50]	TIA, stroke	58/38	64/28-91	5.2		1.0/0.53-1.91[2]
Italy [51]	stroke	35/37	37 (median)/2-50	12.4[3]	<0.001	5.1/1.6-16.3[2]
Italy [48]	TIA, stroke	85/70	43/13	3.8		1.2/0.4-4.0[4]
Italy [26]	CVD, stroke	105 (total)	not given	1.9	ns	
Spain [28]	TIA, stroke	54/50	66/24-88	1.0	ns	
USA [29]	arterial thrombosis[5]	22/7	59	0	ns	
Brazil [32]	arterial thrombosis[5]	35/35	40 (median)/17-56	5.7	0.013	8.4/1.58-45
United Kingdom [54]	coeliac axis thrombosis and splenic infarction	-/1	15	heterozygous[6]		case report

CVD, cerebrovascular disease; TIA, transitory ischemic attack. Age, mean or median and range or ± SD (not available in all cases). OR for comparison with a control group.

[1]p-value, comparison of carrier frequencies in patients and controls ($\chi 2$, [25, 36, 66, 68]; Fisher's exact test, [26]; two sample proportion test, [24]; not given, [27])

[2]incl. patients with additional hereditary risk factors (protein C, protein S, or antithrombin deficiency, FV G1691A mutation)

[3]incl. 2 homozygous patients

[4]subgroup analysis of 96 young patients (age not given by the authors), OR 1.3/0.3-5.0

[5]including CVD, coronary heart disease and peripheral artery disease

[6]user of oral contraceptives

Table 4 The PT mutation in coronary heart disease

Study center	Diagnosis	n (male/female)	Age (years)	Carriers (%)	p^1	OR/95%-CI
USA [25]	MI	-/79	40/18-44	5.1		4.0/1.1-15.1[2]
Brazil [52]	MI	124/96	38 (median)/17-45 60 (median)/46-82	3.2	0.03[3]	
Japan [35]	MI, AP, DVT, PE	93 (total)	57/21-79	0.0	-	
Austria [53]	MI, CHD	79/19	male, 49±9 female 53±812	male, 5.1 female, 5.2	ns	
Italy [26]	MI, AP	90 (total)	not given	3.3	ns	
Spain [28]	MI, unstable AP	74/27	63/34-85	4.0	ns	
USA [70]	MI, DVT, PE	1/-	24	homozygous[4]		case report

CHD, coronary heart disease; PE, pulmonal embolism; AP, angina pectoris. Age, mean or median and range or ± SD (not available in all cases). OR for comparison with a control group.

[1]p-value, comparison of carrier frequencies in patients and controls ($\chi 2$, [25]; Fisher's exact test, [30]; two sample proportion test, [24]; not given, [31])

[2]strong increase of the OR if in combination with metabolic risk factors

[3]same prevalence in older and younger subjects with a trend towards a higher prevalence in women older than 45 years of age, p-value for the entire patient collective

[4]co-inherited with heterozygosity for the FV G1691A mutation

tained, because patient carriers with a possible or not yet determined prothrombotic mutation who have died in consequence of stroke or MI were not represented in the case control study populations investigated [26, 28, 29, 32, 53] (Table 4).

Coexistence of the G20210A and other hereditary prothrombotic risk factors

Co-inheritance of the FV G1691A mutation and protein S, protein C, or antithrombin deficiency leads to a higher risk of thrombosis than single gene defects [55-57]. Therefore, the PT mutation and co-inherited risk factors might also be expected to work synergistically in increasing the thrombotic risk. In few reports individuals carrying both the PT and the FV G1691A mutation, or a further second genetic defect had a higher thrombotic risk than those carrying only the PT mutation [12, 26, 39]. Moreover, these patients were younger at the first thrombotic onset and suffered more frequently from recurrent thrombosis. Most investigators included screening for additional risk factors on subjects suffering thrombotic events as a side aspect in their studies. As the number of PT mutation carriers was small in most cases, patients having a second genetic defect are expected to be rare. Thus, there is no further support for the suggestion of double defects leading to a statistically significantly increased thrombotic risk in such studies [29, 32, 36-38, 40, 41, 58].

Some investigators tried another approach: the selection criterion for patients was the presence of a hereditary risk factor for thrombosis (deficiency of protein C, protein S, or antithrombin, FV G1691A mutation). In most of these studies double mutations increased the thrombotic risk (OR up to 11.3), with a lower age of manifestation or a higher frequency of recurrent thrombosis [33, 59-61]. There is only one study which failed to confirm these data [62], probably due to methodological bias [60] (Table 5).

Screening for the G20210A mutation

Since the heterozygous PT variant is not a very strong genetic risk factor for venous or arterial thrombosis, screening only for this mutation is not recommended. However, the heterozygous PT variant seems to increase the thrombotic risk in the presence of further genetic risk factors such as the FV G1691A mutation. Therefore it should be included in a general screening program which includes established thrombotic risk factors, i.e. FV G1691A mutation, deficiencies of protein C, protein S and antithrombin, as well as hyperhomocysteinemia and at least during childhood, high levels of lipoprotein (a) [64-67]. Due to different ethnical population backgrounds possible variations are unavoidable [68].

Table 5 The PT mutation in thrombophilia patients

Study center	Subjects	n	Age (years)	Carriers (%)
USA [33]	thrombosis patients, FV G1691A carriers	48	not given	10.4
Sweden [59]	members of APC-R families, incl. thrombosis patients	332	not given	-[1]
United Kingdom [61]	DVT patients with hereditary coagulation disorders[2]	101	PT mutation carriers, 49±19 non-carriers, 45±15	7.9 vs. 0.7 in healthy controls, p=0.005 (Fisher's exact test)[3]
France [62]	members of APC-R families, incl. thrombosis patients	288	not given	0
France [58]	PE patients, focus on 2 FV G1691A carriers	200 (total)	male patient, 31 female patient, 26	both patients heterozygous
Italy [63]	Members of a FV G1691A positive family, patients with recurrent DVT	2	male patient, 21 female patient, 23	both patients heterozygous
Germany [60]	Thrombosis patients, FV G1691A carriers	115	28 (median)/18-45	12.2 - OR 3.0 (95%-CI 0.8-11.7)[4]

APC-R, APC-resistance. Age, mean or median and range or ± SD (not available in all cases).

[1]after exclusion of protein S deficient patients: 50% of patients carrying the PT and the FV G1691A mutation suffered a thrombotic event, compared to 27% of patients carrying solely the FV G1691A mutation

[2]either protein S, protein C, or antithrombin deficiency, or FV G1691A mutation

[3]number of thrombotic events significantly higher in PT carriers than in patients with single defects; onset of thrombosis in a younger age if two defects were present

[4]compared with patients carrying solely the FV G1691A mutation (heterozygous); onset of thrombosis in a slightly younger age if the PT mutation was present

Besides the fact that testing for thrombophilic risk factors is performed in symptomatic patients and possibly also in their first-degree family members, there are no data available yet suggesting that general thrombophilia screening prior to surgery or further risk situations such as immobilization, pregnancy, or intake of oral anticontraceptives is recommendable. Whether prophylactic testing of established genetic risk factors for thrombophilia is beneficial in association with risk situations is yet to be evaluated. However, based on a carefully performed clinical examination and exact documentation of the personal or family history (first-degree family members only) of previous thrombosis, early MI, stroke or recurrent fetal wastage in subjects at risk, screening for thrombophilic risk factors prior to situations known to provoke thrombosis should be discussed on an individual basis [64, 65, 69].

How to manage thrombotic events of G20210A carriers

The management of patients with familial thrombophilia comprises acute thrombosis treatment and thromboprophylaxis. Although the immediate treatment of an acute thrombotic event is not significantly different from that of patients without recognized coagulation abnormalities, i.e. thrombolytic therapy, thrombectomy or heparinization, detailed patient long-term management is seriously hampered by the lack of appropriate clinical trials. Thus, until long-term data are available for the treatment of symptomatic individuals with the PT variant, patients should be handled in the same way as those with the FV G1691A mutation, deficiencies of protein C, protein S or antithrombin: asymptomatic subjects of the heterozygous PT variant without a personal history of thrombosis should not receive primary thromboprophylaxis solely because of a heterozygous carrier status of the 20210A allele. In contrast, heterozygous patients who have suffered from thromboembolism and who have no further genetic defects and no positive family history of vascular insults may be given secondary preventive anticoagulant therapy in situations known to provoke thrombosis after withdrawal of oral anticoagulants usually administered for 6 months after the acute thrombotic onset. However, in homozygous symptomatic cases or in patients carrying combined heterozygous genetic defects together with a positive family history of recurrent thrombosis, preventive therapy should be discussed on an individual basis in all risk situations. Long-term anticoagulant therapy is considered in symptomatic subjects in whom thrombotic events have been life-threatening or recurrent [64, 65, 69]. In each case an assessment must be made with respect to the relative benefit conferred by long-term anticoagulant therapy in preventing future thromboembolism versus the potential side effects, costs and inconvenience for the patient.

Conclusions and remarks

In conclusion, available data allows some preliminary statements:

- There are ethnical differences in carrier frequencies of the PT mutation. Prevalences range from 0 to 4% in populations not affected by thrombotic events.
- The PT mutation is a risk factor for venous thrombosis; co-inheritance with other established risk factors (deficiency of protein S, protein C, antithrombin, or the FV G1691A mutation) increases the thrombotic risk, lowers the age of first thrombotic events, and raises the frequency of recurrent thrombotic episodes.
- The PT mutation seems to increase the risk of arterial thrombosis in combination with other established risk factors (e. g. smoking, metabolic risk factors), or at a young age.
- Screening for presence of the mutation should be performed at least in cases of otherwise unexplained thrombotic events in adults, as well as in all thrombotic events during childhood, adolescents or young adults.
- It has to be clarified whether therapeutic strategies for the treatment of patients with hereditary thrombophilia have to be modified for carriers of the PT mutation. Meanwhile treatment should be performed as for thrombosis patients carrying the FV G1691A mutation or other hereditary prothrombotic risk factors.

Less is known about homozygous carriers of the PT mutation, due to the expected carrier frequency of only 0.010-0.014% [12,30]. One existing case report documents a MI followed by DVT and pulmonal embolism in a Mexican male aged 24 years who tested heterozygous for the FV G1691A mutation, and homozygous for the PT mutation [70]. In an epidemiologic study one homozygous patient was identified who suffered DVT, pulmonary embolism and recurrent DVT with late onset of thrombosis at the age of 66 years [29]. More recently, 2 homozygous patients with early onset of stroke [51], and 4 patients with DVT [39] were identified. These findings suggest that homozygosity for the PT mutation is a severe prothrombotic defect, but evidence has yet to be provided.

In case-control studies a bias through elimination of non-survivors of thrombotic events may appear. As carriers of genetic risk factors may suffer more severe thrombotic events than non-carriers, this could lead to misinterpretation of study results and underestimation of a potential risk.

Future prospective studies are needed on subgroups of patients in order to define the risk at different ages, for males and females, in the presence of further hereditary and acquired risk factors.

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Die Bedeutung von Troponin-I-, CK-MB- und Myoglobin-Schnelltests für die Diagnostik bei akuten Herzbeschwerden

Die häufigsten Todesursachen in den Industrieländern sind auf koronare Herzerkrankungen zurückzuführen. Jährlich sind über 14 Millionen Menschen betroffen. Von allen auftretenden Herzinfarkten enden ca. 35 - 45% tödlich, wobei 75% der Patienten sterben, noch bevor sie behandelt werden können. Als Leitsymptom akuter koronarer Herzerkrankungen gilt der plötzlich auftretende Brustschmerz. Derartige Patienten entwickeln in 15 - 20% der Fälle schon innerhalb von 24 Stunden einen akuten Myokardinfarkt (AMI), der bei zahlreichen Patienten auf eine instabile Angina pectoris zurückgeführt werden kann. Eine entsprechende Diagnose bei Patienten mit akuten Brustbeschwerden ist aber zeitaufwendig und zudem teuer und endet oftmals in einem unklaren Befund, da 30 - 40% aller Infarkte klinisch stumm bleiben. Die zur Zeit noch gebräuchlichste Untersuchungsmethode zur Diagnose bei Herzerkrankungen stellt aufgrund seiner raschen Verfügbarkeit das EKG dar. Jedoch nur ca. 50% aller Myokardvorfälle können mittels EKG diagnostiziert werden. Auch die zusätzliche Verwendung klassischer Marker wie z. B. die Enzyme LDH oder CK bieten hier kaum Vorteile, da diese Parameter weder sensitiv genug noch herzmuskelspezifisch sind. Die Neuentwicklung sensitiverer und spezifischerer immunchemischer Marker wie **Troponin-I**, **CK-MB (Masse)** und **Myoglobin** hat hier in letzter Zeit das diagnostische Fenster weiter geöffnet und bringt für die Diagnose, die Verlaufs- und Behandlungskontrolle deutliche Vorteile. Bei verschiedensten klinischen Fragestellungen ermöglichen diese Marker - in Kombination sowie unter Berücksichtigung ihrer zeitlichen Abfolge - sowohl eine sinnreiche Risikoabschätzung als auch eine Differentialdiagnose bei Brustbeschwerden. So weist vorhandenes **Troponin-I** schon nach 1 - 2 Stunden frühzeitig auf eine spezifische Myokardverletzung hin und trägt damit wesentlich zur Abklärung eines klinisch unklaren Befundes bei. Auch läßt sich bei Patienten mit speziellen diagnostischen Problemen wie schwerer Niereninsuffizienz oder Skelettmuskelschädigung – bei normalen Troponin-I Werten - ein akuter Myokardinfarkt ausschließen. Außerdem erweist sich Troponin-I bei Patienten mit instabiler Angina pectoris als Anzeiger für eine ungünstige Prognose, da andere Herzparameter hier keine Aussage ermöglichen. Mit Hilfe des Parameters **Myoglobin** kann der Erfolg einer Thrombolysebehandlung aufgrund seiner sehr schnellen Freisetzung aus geschädigten Muskelzellen, seiner kurzen Halbwertszeit und hohen Sensitivität gut mittels Verlaufsbestimmungen überwacht werden. Zusätzlich gibt dieser Parameter aufgrund der genannten Vorteile wichtige diagnostische Hinweise bei Reinfarkten. Ebenso wie Myoglobin eignet sich auch **CK-MB (Masse)** zur Durchführung von Verlaufskontrollen. Aufgrund des breiteren diagnostischen Zeitfensters und seiner höheren Spezifität trägt CK-MB (Masse) bei Patienten mit klinischen Anzeichen eines AMI, wie z.B. mit bestimmten EKG-Kriterien, als wichtiger Parameter zur Diagnosesicherung bei. Durch die Einbeziehung dieser neuen immunchemischen Marker können innerhalb der modernen Diagnostik akuter ischämischer Herzerkrankungen rechtzeitig unklare Befunde und Reinfarkte abgeklärt, Differentialdiagnosen erstellt und therapeutische Verlaufskontrollen durchgeführt werden.

Die Firma **NOBIS Labordiagnostica** bietet ein **Schnelltest-Programm** zur Diagnostik von ischämischen Herzerkrankungen an. In diesem Bereich stehen durch den Einsatz der Kassettenschnelltests **NobiView-cTn-I, NobiView-Myoglobin** und **NobiView-CK-MB (Masse)** hochempfindliche Bestimmungsmöglichkeiten zur Verfügung. Die Tests sind äußerst einfach durchführbar und liefern die in kürzester Zeit sichere und eindeutige Ergebnisse. Sollten Sie an weiterer Information zu unseren Produkten zur Herzinfarktdiagnostik interessiert sein, fragen Sie bitte nach bei: **NOBIS Labordiagnostica GmbH, Elsässer Str. 18, D-79346 Endingen, Tel. 07642-9055-0 oder Fax 07642-9055-44.**