



The prothrombin gene variant 20210A in venous and arterial thromboembolism

VICENTE VICENTE, ROCIO GONZÁLEZ-CONEJERO, JOSÉ RIVERA, JAVIER CORRAL
Division of Hematology and Clinical Oncology, Hospital General Universitario, Murcia, Spain

ABSTRACT

Several hereditary disorders affecting coagulation factors have been identified as prothrombotic risk factors. Recently, the prothrombin 20210 A/G mutation has been identified as a second important polymorphism involved in venous thrombosis.

This article reviews all published information about this new procoagulant mutation. Our group has been involved in a number of studies about the role and importance of polymorphisms in thromboembolic disease, including the analysis of the prothrombin 20210 A/G mutation. Moreover, an extensive Medline® literature search was made to complete the review using the key words: prothrombin mutation, 20210, venous or arterial thrombosis.

The combination of environmental and genetic risk factors determines the relative risk that any individual has of suffering a thrombotic episode. Some genetic mutations affecting coagulation factors have been described. Recently, Poort *et al.* described a new mutation in the 3'-untranslated region of the prothrombin gene. The prothrombin 20210 G/A mutation, associated with elevated levels of factor II in plasma, significantly increases the risk of developing venous thrombosis. In fact, this polymorphism is the second most important genetic risk factor for venous thrombosis in Caucasian populations. Moreover, and supporting the multifactorial feature of thromboembolic diseases, this mutation greatly increases the possibility of developing a thrombotic episode when combined with other environmental or genetic risk factors. The role of this procoagulant mutation in arterial vascular disease is, however, unclear.

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Key words: prothrombin 20210 A, venous thrombosis, arterial thrombosis

Over the last decade it has been demonstrated that mutations in genes that encode for proteins involved in thrombus formation play an important role in the predisposition to a persistent hypercoagulable state.¹⁻³ Accordingly, several hereditary disorders, particularly those affecting the physiologic anticoagulant systems

(antithrombin III, proteins C and S, and factor V Leiden), have been well established as risk factors for venous thromboembolism.^{4,5} In contrast, there is not yet clear evidence concerning the potential role of these mutations in arterial thrombosis.^{6,7} In a general outpatient population presenting with an objectively documented episode of deep venous thrombosis, the prevalence of heterozygous gene defects affecting these proteins is 10-20%.^{4,5} These disorders occur with roughly equal frequency and are identified with greater prevalence in younger patients and in those with a personal history of recurrent venous thrombotic events or a positive family history.^{4,5} Recently, another gene associated with venous thrombosis, the prothrombin gene, has been identified.

Prothrombin 20210 A and venous thrombosis

Prothrombin is synthesized in the liver as a single chain glycoprotein of 72,000 molecular weight.⁸ This protein and its enzymatically active form, thrombin, are involved in several biological processes. The roles of thrombin as both a procoagulant and an anticoagulant in the process of blood coagulation have been well studied.⁹ Prothrombin is encoded by a 21-kb-pair long gene¹⁰ localized on chromosome 11, band 11p11-q12.¹¹ This gene is organized in 14 exons, separated by 13 introns, with 5' and 3'-untranslated (UT) regions,¹⁰ which may play regulatory roles in gene expression. Several polymorphisms within the human prothrombin gene have been described.⁸ Five of them (located at residues -42, 13, 31, 274, and 389) result in silent substitutions that have no effect on the amino acid sequence of the mature protein. Moreover, some rare genetic defects responsible for hypoprothrombinemia and dysprothrombinemia have been characterized.⁸ All these anomalies are associated with bleeding disorders.

In 1996, Poort *et al.*¹² first investigated the role of the prothrombin gene in venous thrombosis in 28 selected patients with a documented familial history of venous thrombophilia. The strategy for the identification of nucleotide variations in the prothrombin gene was to amplify and sequence all exons, their splice junctions, and the 5'- and 3'-UT regions of the gene. These regions contain the most

Correspondence: Prof. Vicente Vicente, Centro Regional de Hemodonación, Ronda de Garay s/n, 30003 Murcia, Spain.
Phone: international +34-968-341990 - Fax: international +34-968-261914 - E-mail: vvg@fcu.um.es

likely sites for mutations or polymorphisms that would affect transcription, translation or the stability of the translated product. Except for neutral polymorphisms, no nucleotide change was found in the 14 exons and the 5'-UT region of the prothrombin gene. Only one heterozygous nucleotide transition (G to A) at position 20210, the last nucleotide of the 3'-UT region was found in 18% of the patients, but only in 1% of a group of 100 healthy controls. These authors also studied 426 unselected and consecutive outpatients younger than 70 years of age, who were referred for anticoagulant treatment because of a first, objectively diagnosed episode of deep-vein thrombosis. All cases of venous thrombosis were age and sex matched to one healthy control subject according to predefined criteria of the Leiden Thrombophilia Study.¹³ The study revealed a significantly higher prevalence of the prothrombin 20210 A genotype among patients (6.2%) than among control subjects (2.3%). Thus, the relative risk for venous thrombosis associated with the 20210 A allele was 2.8.

Many groups have studied the frequency and the role of this mutation in thromboembolic disease since its discovery. Data obtained from these studies, which were performed in different countries, provided similar results, and confirmed that the 20210 A allele of the prothrombin gene is an important risk factor for venous thrombosis.¹⁴⁻²⁰ This mutation is found in 4-8% of unselected consecutive patients with a first episode of deep venous thrombosis and represent a relative risk of venous thrombosis ranging from 2 to 7 fold in heterozygous carriers of the prothrombin variant (Table 1). All these data contrast with the recent findings of Souto *et al.*²¹ These authors found 20 heterozygous carriers of this mutation out of 116 unrelated patients with venous thromboembolism (17.2%). However, almost half of these patients (54) have presented recurrent events. Addi-

tionally, the authors cannot exclude the possibility that their results were biased because the study included some selected patients from referral Hospitals. Therefore, in order to understand the role and prevalence of this new mutation in thromboembolic disorders it is necessary to know the origin, clinical data and selection criteria of the patients included in each study.

The clinical manifestations of the heterozygous genotype of prothrombin 20210 are those of venous thromboembolism, such as deep vein thrombosis of the legs, pulmonary embolism and superficial thrombophlebitis.^{12,14-17} Visceral vein thrombosis is rarer but quite typical of inherited thrombophilia.^{4,5} Several cases of mesenteric vein thrombosis have been described in patients heterozygous or homozygous for the 20210A allele of the prothrombin gene.²²⁻²⁵ Similarly, the prothrombin gene mutation and risk of retinal vein occlusion have also been related.²⁶ Recently, Martinelli *et al.*²⁷ found a very high prevalence of the prothrombin gene mutation (20%) in patients with idiopathic cerebral vein thrombosis. These data have been confirmed by Eherenforth *et al.*²⁸ On the other hand, it has been suggested that the prothrombin 20210 A/G genotype might lead to an increased thrombotic risk specifically among older patients.¹⁶ However, data from the *Leiden Thrombophilia Study*²⁹ and from De Stefano *et al.*³⁰ indicate that the prothrombin 20210 A/G genotype increases the risk of thrombosis in all ages and in both sexes.

Patients with homozygous defects of naturally occurring anticoagulant systems (antithrombin III, proteins C and S, and factor V Leiden) are usually characterized by severe clinical manifestations, which appear at an early age. So far, only a few cases of homozygous carriers of this mutation have been described and, therefore little clinical information from patients with homozygous prothrombin 20210 A mutation is available. Interestingly, three independent reports identified 5 homozygous patients of the prothrombin mutation who were also carriers of factor V Leiden.^{12,31,32} Other authors have found patients with genotype 20210 A/A without factor V Leiden.³³⁻³⁵ The homozygous genotype 20210 A/A was associated with thrombotic episodes at early ages. Interestingly, in most of these cases, additional environmental risk factors seem to be required to trigger the thrombotic episode. Finally, it is important to point out that no homozygous carriers of this mutation have been found among healthy individuals. Therefore, the available data suggest that homozygosity for the 20210A allele of the prothrombin gene is associated with a severe thrombotic diathesis, albeit more benign, compared with homozygosity for deficiencies of antithrombin III, protein C, or protein S.

An increase in thrombotic tendency has been reported for carriers of combined prothrombotic mutations. Thus, the combination of factor V Leiden and protein C deficiency,³⁶ protein S deficiency^{37,38} or

Table 1. Prevalence of prothrombin 20210A (genotype AG) carriers in unselected patients with venous thrombosis and control subjects. Data obtained from different studies.

Patients		Controls		O.R. [#]	Reference
Genotype AG		Genotype AG			
N	%	N	(%)		
471	6.2	474	2.3	2.8	Poort <i>et al.</i> ¹²
504	5	508	2.6	2	Brown <i>et al.</i> ¹⁵
82	7.3	82	1.2	6.4	Corral <i>et al.</i> ¹⁴
99	7.1	282	1.8	4.2	Hillarp <i>et al.</i> ¹⁶
116	4.3	295	0.7	6.6	Arruda <i>et al.</i> ¹⁷
281	8	850	2.2	3.8	Margaglione <i>et al.</i> ¹⁸
219	5.5	164	1.2	5.4	Cumming <i>et al.</i> ¹⁹
366	4.6	400	1	4.8	Leroyer <i>et al.</i> ²⁰

[#]Odds ratio.

antithrombin III deficiency³⁹ greatly increases the penetrance of thrombotic disease. Additionally, these patients have more severe clinical manifestations and higher risk of recurrence. Consequently, after the discovery of the 20210A mutation in the prothrombin gene as the second more important genetic polymorphism involved in venous thrombosis, the association of this mutation with other prothrombotic mutations was investigated. Makris *et al.*⁴⁰ studied a group of 101 unrelated individuals with a history of venous thromboembolism and either inherited protein C deficiency, protein S deficiency, antithrombin III deficiency or factor V Leiden. Eight percent of these patients were also heterozygous for the prothrombin variant. Although no clear relationship between the prothrombin 20210A allele and protein S-deficiency has been found,⁴¹ and it has been suggested that the effects of the association of this allele with protein C-deficiency are severe,⁴² it is much more interesting to know the effects of the combination of the two main genetic risk factors of venous thrombosis: factor V Leiden and prothrombin 20210A. Despite an initial report showing no association between factor V Leiden and the 20210A mutation,⁴³ subsequent reports found coexistence of the prothrombin 20210 A/G genotype in more than 10% of thrombotic patients with factor V Leiden.^{41,44-48} Interestingly, these studies demonstrated that co-inheritance of the 20210A allele of the prothrombin gene is significantly more common in patients with inherited thrombophilia and recurrent episodes than in patients with only one previous thrombotic episode. The observation that the penetrance of thrombosis in patients with two gene defects is higher than in those with a single defect could be relevant for their clinical management. Additionally, these reports provide further evidence that the venous thrombotic risk is greater in individuals with combined genetic risk factors for familial thrombophilia compared with those who have inherited a single defect. Finally, these findings support the idea that familial thrombophilia is a multiple gene disorder. Accordingly, a careful search for the prothrombin variant should be included in thrombophilia screening programs, particularly in young patients carrying other genetic defects predisposing to thrombosis.

The high prevalence of the prothrombin 20210 A gene among patients with deep vein thrombosis, makes it possible to study its interaction with some common environmental risk factors for thrombosis. It was described that the combined effect of the prothrombin gene mutation and the use of oral contraceptives greatly increased the risk of cerebral vein thrombosis.²⁷ Since both the prothrombin gene mutation and the use of oral contraceptives cause hypercoagulability,^{33,49} their combination probably enhances thrombin generation in the coagulation system. However, although these data are suggestive, more observations are necessary to clarify whether

an interaction exists between the prothrombin gene mutation and current oral contraceptive use, as has been found for the factor V Leiden mutation.⁵⁰ In contrast, preliminary data suggest that the prothrombin variant does not play a major role in the occurrence of thrombosis in primary antiphospholipids syndrome.⁵¹ On the other hand, the risk of venous thrombosis during pregnancy and *post-partum* in hereditary thrombophilia has been well defined.⁵² Recent data also suggest a genetic susceptibility to pregnancy-related thromboembolism in female carriers of the prothrombin 20210 mutation.⁵³ Further studies should define whether the presence of the prothrombin 20210 A allele could also increase the risk of developing thrombosis in those individuals with other well established environmental risk factors such as trauma, immobilization, or surgery.

Ethnic distribution of prothrombin 20210 A

The geographic distribution of the 20210 G/A mutation of the prothrombin gene shares common features with the most commonly observed prothrombotic polymorphism, i.e. factor V Leiden. Both mutations are mainly restricted to Caucasian populations. The allele frequency of factor V Leiden varies from 1% to 8% in Caucasians, whereas among Africans, Chinese, Japanese and native North and South Americans, this polymorphism is absent.⁵⁴ The frequency of the 20210 A allele of the prothrombin gene also varies in Caucasian populations, ranging from 1.2% to 4%.⁵⁵ However, the mutation was found in only 1 of 444 African Americans,⁵⁶ and 2 individuals from 295 Amerindians from Brazil.¹⁷ This mutation has not been found in Somalians,⁵⁷ people of West African origin,⁵⁸ Amazonian Indians,¹⁷ or Japanese subjects.^{59,60} Interestingly, a recent report by Zivelin *et al.*⁶¹ which analyzed the genetic linkage of several polymorphisms of the prothrombin gene in different populations, demonstrated the single genetic origin of the 20210A prothrombin variant which, similarly to factor V Leiden, probably occurred after the divergence of African from non-African and of Caucasian from Asian subpopulations.

The heterogeneity in the prevalence of the 20210 A allele in Caucasian populations was explained by Rosendaal *et al.*⁵⁵ as a result of geographic differences, showing a North-South gradient in the prevalence of this mutation in European population. However, this study considers that Paris and Vienna belong to the South of Europe, while these regions are included in the North of Europe in other studies showing a North-South genetic gradient for other mutations.⁶² Moreover, 57.9% of the individuals from the South of Europe included in this study (1081 subjects) were enrolled in Tel Aviv, Israel. The original ancestry of these subjects could be diverse and therefore, they might not have been very representative of the population from the South of Europe. Finally, a

gradient has already been found for the prevalence of the factor V Leiden in Europe, but the distribution is different for factor V Leiden and 20210A mutation: in Northern Europe there was a higher prevalence of factor V Leiden but lower prevalence of 20210A, something that is difficult to explain in the context of genetic fitness and selection.^{54,55} Considering all data available, the overall prevalence of the 20210A allele in Caucasian populations appears to be around 2%, with small variations between countries or regions that could be explained by the size of the samples.

Prothrombin 20210 A and plasma factor II levels

The underlying mechanism by which prothrombin 20210A may contribute to thrombosis is still unknown. However, an association has been demonstrated between the presence of the 20210 A allele and an elevated prothrombin level.^{12,31,33,40,57,63} This increased plasma level of factor II has been shown to be a risk factor for venous thrombosis.¹² Interestingly, the plasma levels of proteins such as factor VII or fibrinogen seem to be raised by genetic mutations in their respective genes, and such increases have been identified as risk factors for arterial disease.^{62,64} Nevertheless, it remains to be established whether there is a direct causal relationship between the existence of the single G to A transition at position in the 3'-UT region of the prothrombin gene and the increased factor II plasma level, or whether the mutation is in linkage disequilibrium with another sequence variation with a transcription control role in the prothrombin gene capable of affecting factor II gene expression.

Finally, it is still unclear how elevated prothrombin plasma levels may stimulate the generation of venous thrombi. Recently, a significantly increased endogenous thrombin potential was found in heterozygous, and even more so, homozygous carriers of the 20210A prothrombin mutation compared with age-matched controls.³³ Therefore, on stimulation of the coagulation cascade, larger amounts of thrombin could be generated in patients with the 20210A allele; this appears to be the same result as the effect of APC-resistance, or deficiencies of protein C and protein S.⁶⁵

Prothrombin 20210 A and arterial thrombosis

Although many of the genetic variations of coagulation proteins are well established as being involved in venous thrombosis, new inherited defects which might be associated with arterial thrombosis have recently been described: fibrinogen,⁶⁴ factor VII,⁶⁶ factor V Leiden⁶⁷ and homocysteine.⁶⁸ Currently, the role of the prothrombin variation for arterial vascular disease is unclear.^{14,17,34,57,69-73} Rosendaal *et al.*⁶⁹ showed that prothrombin 20210 A increases the risk of myocardial infarction in women aged 18 to 44 years.

The mutation was found in 1.6% of 381 healthy control women, and in 5.1% of 79 women who had a myocardial infarction at a young age. Thus, carriers of prothrombin 20210 A have a 4-fold increased risk of myocardial infarction. The risk associated with the prothrombin mutation was particularly high when some other major risk factors, such as smoking or obesity, were also present. This result of a striking synergy with other major cardiovascular risk factors, was similar to that previously described by Rosendaal *et al.*⁶⁷ for the association between factor V Leiden and myocardial infarction in the same group of young women. In another study, the prothrombin 20210A mutation was found in 5.1% of 98 patients with coronary heart disease but in only 1.96% of healthy neonates.⁷¹ Recently, Doggen *et al.*⁷² examined the presence of the prothrombin mutation in 540 men with a first myocardial infarction before the age of 70 years. The mutation was found in 1.8% of the patients, and 1.2% of the control group consisting of 646 men matched by age. Thus, the mutation did not significantly increase the risk of developing myocardial infarction in men (Odds ratio 1.5). However, the combination of the prothrombin 20210A allele with smoking or obesity, multiply, according to this study, the possibility of suffering from myocardial infarction.⁷²

On the other hand, Corral *et al.*,¹⁴ using a different strategy, carried out two case/control studies both in unselected, consecutive survivors of acute cerebrovascular events (104 patients), and in patients diagnosed as having acute coronary syndromes (101 patients). The control subjects were matched for sex, race and age. Since genetic and environmental factors could act additively or synergistically to determine the individual's risk of arterial thrombosis, the strategy of these authors for identifying the 20210 A mutation as a putative risk factor predisposing to arterial disease, was to avoid overrepresentation of classic vascular risk factors in the cerebrovascular and cardiovascular patients compared to controls. Thus, they tried to approximate risk factors between each case patient and a single control who was age, race and sex matched. The results of this study showed that the prevalence of the prothrombin 20210 A allele did not differ significantly in cerebrovascular or cardiovascular patients with respect to controls.¹⁴ However, a detailed analysis of the results showed that carriage of the prothrombin 20210 A gene was associated with a slightly, although not significantly, increased risk of myocardial infarction (relative risk 2.0) with respect to controls with the same environmental risk factors. Among the recognized risk factors for stroke, migraine has been considered an independent risk factor for ischemic cerebrovascular disease, particularly among young women, and in the absence of other well established risk factors.^{74,75} Iniesta *et al.*⁷⁶ evaluated the synergism between migraine and several prothrombotic genetic risk factors, including factor II 20210A. This

group concluded that the allele does not seem to be associated with the development of ischemic cerebrovascular disease in patients with personal antecedents of migraine.⁷⁶ Additionally, two recent studies did not find a significantly increased prevalence of the mutation in patients with arterial vascular disease (coronary or cerebrovascular disease).^{57,77} Ferraresi *et al.*⁵⁷ observed that the frequency of the prothrombin gene mutation was not increased in 195 patients with arterial disease. In agreement, Franco *et al.*,⁷⁰ studying 263 consecutive patients with premature coronary artery disease (<50 years old), found that the prothrombin 20210 A allele was associated with an increased risk of coronary disease (odds ratio 2.7), but the data were not statistically significant. In contrast, when De Stefano *et al.*³⁴ investigated 72 highly selected young patients (<50 years old), with documented ischemic stroke, and without risk factors such as diabetes, hypertension, or hyperlipidemia, they found 7 heterozygotes (9.7%) and 2 homozygotes (2.7%) for the mutant prothrombin. The relative risk associated with carriership of the prothrombin-gene mutation was estimated as 5.1. It was quite surprising to find 2 homozygous carriers of the mutation. Accordingly, these authors suggested that the homozygous genotype seems to predispose strongly to ischemic stroke, but further investigations on larger series of patients are advisable. Although there is no demonstrated reasons to assume that risk factors in the young differ qualitatively from those in middle-age and older adults, they may differ in their strengths.⁷⁸ This observation and the discrepancies among the data available so far, indicate that further prospective studies including large groups of unselected patients with arterial thrombosis are needed to clarify the specific interactions between atherogenic and genetic risk factors of thrombosis, including prothrombin 20210A allele gene.

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VV, RG-C, JR, and JC contributed equally to this review. JC is a Post-doctoral Fellow of the Universidad of Murcia. RG-C is a fellow of Caja Murcia.

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