The Prevalence of the Prothrombin (*F2*) 20210G>A Mutation in a Cohort of Sri Lankan Patients with Thromboembolic Disorders

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Abstract Prothrombin (*F2*) 20210G>A [rs1799963 G>A] mutation is a genetic variant which predisposes to inherited thrombophilia. Highest prevalence of this rare mutation has been reported among Caucasian and Mediterranean populations with thrombophilic conditions compared to healthy controls. It is absent or occurs in a very low frequency in both thrombophilic patients and healthy controls of most South Asian populations. A previous study has demonstrated that the mutant allele is absent among Sri Lankan healthy controls. This study was conducted to determine the prevalence of the *F2* 20210G>A mutation among Sri Lankan patients with thrombo-embolic disorders. *F2* 20210G>A mutation analysis was carried out on 825 patients. These included 374 with arterial thrombo-embolic disorders, 303 with venous thromboembolic disorders (VTE) and 148 with pregnancy related complications. Genotyping was done using polymerase chain reaction followed by restriction fragment length polymorphism. The overall prevalence of the individuals detected with the mutation was 0.8 % (7/825) with a mutant allele frequency of 0.4 % (7/1,650), and all were heterozygotes. Further classification according to the types of thrombotic events showed a prevalence of 0.5 % (2/374), 1.3 % (4/303), and 0.7 % (1/148) respectively, in the three groups with arterial thrombosis, VTE and pregnancy complications. The respective mutant allele frequencies in the three different groups were 0.3 % (2/748), 0.7 % (4/606) and 0.3 % (1/296). Although these figures are lower

than that of Caucasian and Mediterranean populations, they are relatively higher compared to other South Asian populations. Therefore, the *F2* 20210G>A mutant allele is not entirely absent among Sri Lankan patients with thrombo-embolic disorders.

Keywords Thrombophilia · Venous thrombo-embolic disorders · Prothrombin · *F2* 20210G>A · Prevalence

Introduction

Thrombophilia is a multifactorial disorder of inappropriate clot formation which can be caused due to an interaction of genetic, acquired, and/or circumstantial predisposing factors. Venous thromboembolism (VTE) most commonly manifests as deep vein thrombosis, which may progress to pulmonary embolism if the clot dislodges and travels to the lungs. Other thrombotic manifestations include thrombosis of the cerebral or visceral veins, arterial thrombotic events such as cerebrovascular accidents, myocardial infarctions and pregnancy related complications such as pre-eclampsia and recurrent pregnancy losses [1–3]. Hypercoagulability that causes thrombophilia can be either inherited or acquired. The substitution of nitrogenous base G (Guanine) by A (Adenine) at position 1691 (1691G>A) in the Factor V gene [factor V Leiden] (rs6025), the substitution of G to A at position 20210 (20210G>A) (rs1799963) in the Prothrombin (*Factor II/F2*) gene and the homozygous substitution of C (Cytosine) by T (Thymine) at position 677 (677C>T) (rs1801133) in the 5, 10-methylenetetrahydrofolate reductase (*MTHFR*) gene are the most common causes of inherited thrombophilia [1, 4]. In most inherited thrombophilias, impaired neutralization of thrombin or a failure to control the generation of thrombin causes thrombosis [1].

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Prothrombin encoded by the *F2* gene, is a central regulatory component of the coagulation cascade which in its active form, thrombin, performs both procoagulant and anticoagulant roles [5, 6]. The prothrombin *F2* 20210G>A mutation located at the 3' cleavage site of the mRNA of the *F2* gene results in elevated plasma prothrombin levels and an increased risk of thrombosis [6]. This variant has been shown to directly influence a variety of processes related to prothrombin mRNA metabolism such as prothrombin pre-mRNA cleavage, polyadenylation, mRNA stability and translation [6–10].

According to a review by Jadaon [11], the prevalence of the individuals having *F2* 20210G>A mutation, ranges from 3 to 17 % in patients with VTE and 1 to 8 % in healthy controls among Caucasians [11]. High prevalence of this mutation has also been reported in populations living close to Europe, mainly in the Middle East and North Africa. The prevalence in these countries is similar to that reported in South European countries which is approximately 3 to 24 % in VTE patients and 1 to 12 % in the general population [11]. Previous studies have reported the absence of this mutation among healthy controls in Asian populations in China, Japan, Korea, Mongolia, Taiwan, Indonesia, Burma, Cambodia, Thailand, Taiwan, Vietnam, Hong Kong, Pakistan and Bangladesh [11, 12, 15–19, 21–25], while in India it was reported to be between 0 to 0.6 % [11, 19, 22, 25–29]. Among the VTE patients in China, Korea, and Taiwan, the prevalence of the mutation carriers was 0 % [11, 12, 15–17, 19–24]. Even among the Indian VTE patients, the prevalence was 0 % [11, 19, 22, 25–29]. Hence it is likely that the *F2* 20210G>A mutation occurs in a very low frequency in Asian and other non Caucasian populations [11, 12]. A study conducted by Dissanayake et al. [13] in 2009 reported that the *F2* 20210A mutant allele is absent among healthy Sri Lankans. The prevalence of the individuals with *F2* 20210G>A mutation among Sri Lankan thrombophilic patients has not been reported previously. Hence this study was undertaken to determine the prevalence of the individuals with the *F2* 20210G>A mutation along with the mutant allele frequency among Sri Lankan patients with thrombo-embolic disorders referred for thrombophilia screening.

Methodology

Patients who were referred to our unit from February 2006 to April 2013 for *F2* 20210G>A mutation testing due to arterial thrombotic events [cerebral and myocardial infarctions], venous thromboembolic events [deep vein thrombosis, pulmonary embolism, thrombosis of the cerebral or visceral veins], and pregnancy complications [recurrent pregnancy losses, placental abruption, and pre-

eclampsia] were included in this study. All the patients have provided written informed consent for testing.

Genomic DNA was extracted using Wizard genomic DNA purification kit (Promega, USA) according to the manufacturer's protocol. Extracts were subjected to gel electrophoresis using 1 % agarose gel to confirm the presence of DNA. The mutation was detected using the routine polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method carried out at the human genetics unit. It was a duplex PCR for the *F2* 20210G>A and *FV* 1691G>A mutations adapted from a previously described method by Koksai et al. in 2007 [14]. The PCR was followed by RFLP assay based on *MnII* endonuclease digestion.

The PCR reaction was performed in a total volume of 25 µl containing 1× buffer (Promega, USA), 2.0 mmol/l MgCl₂, 0.2 mmol/l dNTPs, 0.4 µmol/l of each *F2* primer, 0.8 µmol/l of each *FV* primer and 5 units of *Taq* DNA polymerase (Promega, USA). The PCR cycling conditions included initial denaturation of 5 min at 94 °C followed by 35 cycles with 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C and a final extension of 10 min at 72 °C as described in the original article [14]. This PCR amplifies a 169-bp product from exon 10 of *FV* and a 221-bp product from the 3'untranslated region of *F2* gene [14]. From the PCR product, 10 µl was digested with 5 units of *MnII* in 1× solution of its buffer. After the digestion, the *F2* amplicon with the mutant allele was supposed to remain undigested while the wild type allele provides one digestion site to yield two products of 29-bp and 192-bp. The *FV* amplicon with the wild type allele was digested into three products of 17-bp, 37-bp and 115-bp while the mutant allele containing amplicon was digested into two: 17-bp and 152-bp. Digested amplicons were visualized after gel-electrophoresis on a 3 % agarose gel incorporated with ethidium bromide. A gel image with the wild type and mutant bands of *F2* and *FV* is shown in Fig. 1. The positive controls in each genotyping batch had been sequenced by Sanger sequencing and confirmed. The two double heterozygotes for the *FV*1691 G>A and *F2* 20210 G>A mutations were also confirmed by Sanger sequencing.

Results

The total number of patients tested was 825. Of them, 340 (41.2 %) were males and 485 (58.8 %) were females. The ethnicity of the study population comprised of Sinhalese (85 %), Tamils (6 %) and Moors (5 %). In 4 % of the patients the ethnicity was not documented. The ages of the patients ranged from 1 to 77 years. The distribution of the patients tested, according to the indication for referral is shown in Table 1.

Fig. 1 Ethidium bromide incorporated 3 % agarose gel picture showing the products after duplex PCR–RFLP analysis of wild type and mutant alleles of *F2* 20210G>A and *FV* 1691G>A. Lane 1 A

patient who is a double heterozygote for both mutations, Lane 2 *FV* 1691G>A positive control (heterozygote), Lane 3 *F2* 20210G>A positive control (heterozygote), Lane 4 negative control, Lane B blank

The prevalence of individuals with the *F2* 20210G>A mutation in this cohort was 0.8 % (7/825). Six of the positive cases were Sinhalese and one case was a Moor. All were heterozygotes for the mutation and no homozygotes were found. Among them, 0.6 % (5/825) had only the *F2* 20210 G>A mutation while 0.2 % (2/825) were double heterozygotes for the *FV* 1,691 G>A and *F2* 20210 G>A mutations. The mutant allele frequency in this cohort of patients was 0.4 % (7/1650). The results were further analyzed after categorizing the patients into three groups according to the types of thrombotic events. It showed that among the patients with arterial thrombosis, the prevalence of the individuals who have the mutation was 0.5 % (2/374); 1.3 % (4/303) in those with VTE. Among the patients with pregnancy related complications, it was 0.7 % (1/148). The respective mutant allele frequencies in the three groups were 0.3 % (2/748), 0.7 % (4/606) and 0.3 % (1/296). Screening of asymptomatic family members of some of the index patients was also carried out. The clinical details of the patients who were heterozygotes for the *F2* 20210G>A mutant allele and the results of asymptomatic family members who were tested are summarized in Table 2.

Discussion

In patients with thrombo-embolic events, inherited thrombophilia is suspected when the patient has recurrent or life-threatening venous thromboembolism, family history of venous thrombosis, thrombo-embolic events in patients

aged less than 45 years of age, without apparent acquired risk factors or in females with a history of multiple miscarriages, stillbirths, or both. Although inherited thrombophilia is often suspected in such cases, both acquired and genetic factors may frequently co-interact, thus making it difficult to decide whether the patient should undergo screening tests for inherited factors such as *F2* and *FV* mutations [30].

The substitution of adenine for guanine at nucleotide position 20210 of the Prothrombin gene (*F2* 20210G>A) was first described as a cause of inherited thrombophilia by Poort et al. [31] in 1996. They described the 20210G>A mutation found in the 3' untranslated region of the Prothrombin gene as a moderate risk factor for venous thrombosis. The risk of developing VTE is increased even in the presence of a heterozygous *F2* 20210G>A mutation. This mutation is found to be associated with an increased level of plasma prothrombin. The mechanism which up-regulates the plasma prothrombin level is not clearly known [31]. This increased level of prothrombin promotes the generation of thrombin [30, 32, 33], which in turn increases the risk of developing venous thrombosis by almost threefold in patients of all ages [31]. This risk is slightly lower than that for *FV* 1691G>A [*FV* Leiden] heterozygotes who have a 3–8 fold increased risk and significantly lower than that for *FV* Leiden homozygotes who have an almost 80 fold increased risk [34]. Although *F2* 20210G>A is one of the common genetic risk factors causing inherited thrombophilia in white Caucasians, it is less reported in Asians and Africans [30]. A large meta-analysis by Gohil et al. [35] confirmed a significant

Table 1 Distribution of patients according to the indication for referral

Indication		Number (%)	Total
Arterial	Cerebro vascular accidents	309 (37.4)	374 (45.3 %)
	Myocardial infarctions	40 (4.8)	
	Other arterial thrombosis (e.g. Gangrene)	25 (3.0)	
VTE	Deep vein thrombosis (DVT)	151 (18.3)	303 (36.7 %)
	Pulmonary embolism (PE)	28 (3.4)	
	DVT + PE	13 (1.6)	
	Cerebral venous thrombosis	29 (3.5)	
	Abdominal vein thrombosis	59 (7.2)	
	Other (e.g. neck/retinal vein thrombosis)	23 (3.8)	
Other (pregnancy related complications)	Intrauterine deaths	2 (0.2)	148 (17.9 %)
	Intrauterine growth restriction	1 (0.1)	
	Neonatal deaths	1 (0.1)	
	Bad obstetric history	2 (0.2)	
	Secondary subfertility	2 (0.2)	
	Single miscarriage	7 (0.8)	
	Two miscarriages	1 (0.1)	
	Recurrent miscarriages (>2)	130 (15.8)	
	Two failed in vitro Fertilizations	1 (0.1)	
	Pre-eclampsia	1 (0.1)	
Total		825 (100)	825 (100 %)

association between VTE and *F2 20210G>A* in Caucasian populations. According to their report, the smaller sample sizes tested in non-European populations make definitive conclusions about the genetic aetiology of VTE less reliable in those groups [35]. Another pooled analysis of several case control studies by Emmerich et al. [34] has demonstrated a significant increase in venous thrombosis in double heterozygotes for *FV 1691G>A* and *F2 20210G>A* mutations than in those with a single mutation. Furthermore, it was reported that the double heterozygotes had thrombosis at a significantly younger age compared to the rest [34]. Other than *FV Leiden*, another mutation: *F2 19911A>G* found in the same gene has also been described as a risk factor which slightly increases the risk of venous thrombosis when co-exists with the *F2 20210G>A* mutation [36].

Studies conducted among different racial groups have confirmed varying prevalences of the individuals with *F2 20210G>A* mutation. The highest prevalence of *F2 20210G>A* mutation has been reported among Caucasians of European origin and Mediterranean populations living close to Europe. Furthermore, the prevalence of the mutation in those populations is higher among patients with thrombo-embolic disorders than in healthy controls [11, 12]. However, in many Asian countries it is reported to be absent in both the healthy controls as well as among patients with thrombo-embolic disorders [11, 12, 15–19, 21–25]. Even in India, only one study has reported a

prevalence of 0.6 % among healthy controls [22], while some other studies have reported it as 0 % [11, 19, 25–29]. The mutation has not been reported among Indian patients with venous thrombo-embolism [11, 19, 22, 25–29]. These studies suggest that the *F2 20210G>A* mutation may not be a significant genetic risk factor predisposing to inherited thrombophilia in those populations.

In 2009, a study was conducted by Dissanayake et al. to determine the *F2 20210G>A* mutant allele frequency among healthy controls in the Sri Lankan population. They reported that the mutation was absent among healthy controls across all the three ethnic groups (Sinhala, Tamil and Moor) in Sri Lanka [13]. The present study was undertaken to determine the prevalence of the *F2 20210G>A* mutation among Sri Lankan patients with thromboembolic disorders. In contrast to most reports from other Asian populations, we found that the mutation was not entirely absent among these patients as 0.8 % were heterozygotes for the mutation with a mutant allele frequency of 0.4 %. If the VTE group alone is considered, the prevalence of Sri Lankan VTE patients having this mutation was 1.3 % (with a mutant allele frequency of 0.7 %). This is higher than almost all the VTE groups that have been studied in South and Southeast Asia. The arterial thrombosis group in this study had 2 (0.5 %) patients with a heterozygous mutation. Although significantly associated with VTE [31], the role of *F2 20210G>A* in arterial vascular diseases is not very clear [37, 38]. Rosendaal et al.

Table 2 Clinical details of patients who were heterozygotes for the *F2* 20210G>A and *FV* 1691G>A mutant alleles along with the screening results of asymptomatic family members

Patient	Indication for referral	Thrombotic event (venous/arterial/Other)	Sex	Age (years)	<i>F2</i> 20210G>A genotype	<i>FV</i> 1691G>A genotype	Family screening
216	Cerebro vascular accident	Arterial	Male	34	GA	GG	Father (aged 65 years) was a heterozygote.
737	Cerebro vascular accident	Arterial	Male	42	GA	GA	Parents were not available for testing.
821	Abdominal vein thrombosis	Venous	Female	30	GA	GG	Parents were not available for testing.
840	Deep vein thrombosis	Venous	Female	51	GA	GG	Mother (aged 71 years) was homozygous for the normal allele. Father was not available for testing. Two sons (aged 35 years and 30 years) and the daughter (aged 28 years) were heterozygotes. They were clinically asymptomatic.
894	Abdominal vein thrombosis	Venous	Female	39	GA	GG	Parents were not available for testing.
166	Deep vein thrombosis	Venous	Male	38	GA	GA	Parents were not available for testing.
854	Pregnancy complications	Other	Female	26	GA	GG	Mother (aged 54 years) and sister (aged 28 years) were heterozygotes. They were clinically asymptomatic.

[39] showed that it increases the risk of myocardial infarction in young women aged 18 to 44 years [38, 39]. Such an association was not detected among men [38, 40]. In our study, both the arterial thrombotic patients detected with the *F2* 20210G>A mutation were males who had cerebro-vascular accidents. One of them was a double heterozygote for the *F2* 20210G>A and *FV* Leiden mutations. Although the other patient did not have the *FV* Leiden mutation, there is a possibility that he may have some other genetic risk factors known to increase the risk of arterial thrombosis such as polymorphisms in fibrinogen [41] and Factor VII genes [42] or elevated levels of homocysteine due to mutations in genes regulating the methionine-homocysteine pathway [43].

Findings of this study suggest that the *F2* 20210G>A screening should not be abandoned from Sri Lankan test panels for genetic thrombophilia as it is not completely absent among Sri Lankan VTE patients. More case-control studies are required in order to confirm the significance of *F2* 20210G>A as a genetic risk factor among Sri Lankans. Furthermore, the prevalence, allele frequencies and the association of other genetic risk factors i.e. *MTHFR* 677C>T, *FV* 1691G>A, *FV* 4070A>G, and *F2* 11991G>A polymorphisms should also be studied in order to determine the mutations that should be selectively incorporated into the Sri Lankan genetic thrombophilia test panels.

References

1. Seligsohn U, Lubetsky A (2001) Genetic susceptibility to venous thrombosis. *New Eng J Med* 344:1222–1231
2. Varga EA, Kujovich JL (2012) Management of inherited thrombophilia: guide for genetics professionals. *Clin Genet* 81:7–17
3. McKusick VA, Hamosh A, Kniffin CL, Bocchini CA, O'Neill MJF(1986-2012) Coagulation factor II; *F2* OMIM no. 176930. <http://www.omim.org/entry/176930?search=prothrombin%20G20210A&highlight=g20210a%20prothrombin#editHistory-shutter>. Accessed 18/07/2013.
4. De Stefanov V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, Rossi E, Leone G (2013) The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. *New Eng J Med* 341:801–806
5. Spronk HM, Govers-Riemslog JW, Cate H (2003) The blood coagulation system as a molecular machine. *Bioessays* 25:1220–1228
6. Sachchithanathan M, Stasinopoulos SJ, Wilusz J, Medcalf RL (2005) The relationship between the prothrombin upstream sequence element and the G20210A polymorphism: the influence of a competitive environment for mRNA 30-end formation. *Nucleic Acids Res* 33:1010–1020
7. Gehring NH, Frede U, Neu-Yilik G, Hundsdoerfer P, Vetter B, Hentze MW, Kulozik AE (2001) Increased efficiency of mRNA 30 end formation: a new genetic mechanism contributing to hereditary thrombophilia. *Nat Genet* 28:389–392
8. Carter AM, Sachchithanathan M, Stasinopoulos S, Maurer F, Medcalf RL (2002) Prothrombin G20210A is a bifunctional gene polymorphism. *Thromb Haemost* 87:846–853

9. Ceelie H, Spaargaren-van Riel CC, Bertina RM, Vos HL (2004) G20210A is a functional mutation in the prothrombin gene; effect on protein levels and 30-end formation. *J Thromb Haemost* 2:119–127
10. Pollak ES, Lam HS, Russell JE (2002) The G20210A mutation does not affect the stability of prothrombin mRNA in vivo. *Blood* 100:359–362
11. Jadaon MM (2011) Epidemiology of Prothrombin G20210A mutation in the Mediterranean region. *Mediterr J Hematol Infect Dis* 3(1):e2011054. doi:10.4084/MJHID.2011.054
12. Franco RF, Santos SEB, Elion J, Tavella MH, Zago MA (1998) Prevalence of the G20210A polymorphism in the 3'-untranslated region of the Prothrombin gene in different human populations. *Acta Haematol* 100:9–12
13. Dissanayake VHW, Weerasekera LY, Gammulla CG, Jayasekara RW (2009) Prevalence of genetic thrombophilic polymorphisms in the Sri Lankan population - implications for association study design and clinical genetic testing services. *Exp Mol Pathol* 87:159–162
14. Koksai V, Baris I, Ozdal E (2007) Primer-Engineered multiplex PCR-RFLP for detection of MTHFR C677T, Prothrombin G20210A and factor V Leiden mutations. *Exp Mol Pathol* 83:1–3
15. Jun ZJ, Ping T, Lei Y, Li L, Ming SY, Jing W (2006) Prevalence of factor V Leiden and prothrombin G20210A mutations in Chinese patients with deep venous thrombosis and pulmonary embolism. *Clin Lab Haematol* 28:111–116
16. Lu Y, Zhao Y, Liu G, Wang X, Liu Z, Chen B, Hui R (2002) Factor V gene G1691A mutation, prothrombin gene G20210A mutation, and MTHFR gene C677T mutation are not risk factors for pulmonary thromboembolism in Chinese population. *Thromb Res* 106:7–12
17. Sakuma M, Shirato K (2003) The epidemiology of acute pulmonary thromboembolism. *Nihon Rinsho* 61:1706–1712
18. Miyata T, Kawasaki T, Fujimura H, Uchida K, Tsushima M, Kato H (1998) The prothrombin gene G20210A mutation is not found among Japanese patients with deep vein thrombosis and healthy individuals. *Blood Coagul Fibrinolysis* 9:451–452
19. Hessner MJ, Luhm RA, Pearson SL, Edean DJ, Friedman KD, Montgomery RR (1999) Prevalence of prothrombin G20210A, factor V G1691A (Leiden), and methylenetetrahydrofolate reductase (MTHFR) C677T in seven different populations determined by multiplex allele-specific PCR. *Thromb Haemost* 81:733–738
20. Chang JD, Hur M, Lee SS, Yoo JH, Lee KM (2008) Genetic background of nontraumatic osteonecrosis of the femoral head in the Korean population. *Clin Orthop Relat Res* 466:1041–1046
21. Kim YH, Kim J (2007) The 2007 John Charnley award. Factors leading to low prevalence of DVT and pulmonary embolism after THA: analysis of genetic and prothrombotic factors. *Clin Orthop Relat Res* 465:33–39
22. Rees DC, Chapman NH, Webster MT, Guerreiro JF, Rochette J, Clegg JB (1999) Born to clot: the European burden. *Br J Haematol* 105:564–566
23. Shen MC, Lin JS, Tsay W (2000) Protein C and protein S deficiencies are the most important risk factors associated with thrombosis in Chinese venous thrombophilic patients in Taiwan. *Thromb Res* 99:447–452
24. Lin JS, Shen MC, Tsay W (1998) The mutation at position 20210 in the 3'-untranslated region of the prothrombin gene is extremely rare in Taiwanese Chinese patients with venous thrombophilia. *Thromb Haemost* 80:343
25. Newton LJ, Krishnan A, Parapia LA (1999) Born to clot: the European burden. *Br J Haematol* 107:213
26. Kumar SI, Kumar A, Srivastava S, Saraswat VA, Aggarwal R (2005) Low frequency of factor V Leiden and prothrombin G20210A mutations in patients with hepatic venous outflow tract obstruction in northern India: a case-control study. *Indian J Gastroenterol* 24:211–215
27. Ghosh K, Shetty S, Madkaikar M, Pawar A, Nair S, Khare A, Pathare A, Jijina F, Mohanty D (2001) Venous thromboembolism in young patients from western India: a study. *Clin Appl Thromb Hemost* 7:158–165
28. Angeline T, Bently H, Hawk AB, Mannes RJ, Mokashi HA, Jeyaraj N, Tsongalis GJ (2005) Prevalence of the factor V G1691A and the factor II/prothrombin G20210A gene polymorphisms among Tamilians. *Exp Mol Pathol* 79:9–13
29. Garewal G, Das R, Ahluwalia J, Mittal N, Varma S (2003) Prothrombin G20210A is not prevalent in North India. *J Thromb Haemost* 1:2253–2254
30. Seligsohn U, Lubetsky A (2001) Genetic susceptibility to venous Thrombosis. *New Eng J Med* 344:1222–1231
31. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 88:3698–3703
32. Butenas S, van't Veer C, Mann KG (1999) "Normal" thrombin generation. *Blood* 94:2169–2178
33. Smirnov MD, Safa O, Esmon NL, Esmon CT (1999) Inhibition of activated protein C anticoagulant activity by prothrombin. *Blood* 94:3839–3846
34. Emmerich J, Rosendaal FR, Cattaneo M, Margaglione M, De Stefano V, Cumming T, Arruda V, Hillarp A, Reny JL et al (2001) Combined effect of factor V Leiden and Prothrombin 20210A on the risk of venous thromboembolism-pooled analysis of 8 case-control studies including 2,310 cases and 3,204 controls. *Thromb Haemost* 86:809–816
35. Gohil R, Peck G, Sharma P (2009) The genetics of venous thromboembolism: A meta-analysis involving ~1,20,000 cases and 1,80,000 controls. *Thromb Haemost* 102:360–370
36. Chinthammitr Y, Vos HL, Rosendaal FR, Doggen CJM (2006) The association of prothrombin A19911G polymorphism with plasma prothrombin activity and venous thrombosis: results of the MEGA study, a large population-based case-control study. *J Thromb Haemost* 4:2587–2592
37. Corral J, González R, Lozano ML, Rivera J, Heras I, Vicente V (1997) The venous thrombosis risk factor 20210 A allele of the prothrombin gene is not a major risk factor for arterial thrombotic disease. *Br J Haematol* 99:304–307
38. Vicente V, Gonzalez-Conejero R, Rivera J, Corral J (1999) The prothrombin gene variant 20210A in venous and arterial thromboembolism. *Haematologica* 84:356–362
39. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL (1997) A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood* 90:1747–1750
40. Doggen CJ, Cats VM, Bertina RM, Rosendaal FR (1998) Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. *Circulation* 97:1037–1041
41. Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G, Cambou J-P, Scarabin P-Y, Bara L, Green F, Cambien F (1996) β fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. *Circulation* 93:440–449
42. Iacovello L, Castelnovo A, Knijff P, D'Orazio A, Amore C, Arboretti R, Kluff C, Donati MB (1998) Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. *New Eng J Med* 338:79–85
43. D'Angelo A, Selhub J (1997) Homocysteine and thrombotic disease. *Blood* 90:1–11