Thrombophilia

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Thrombophilia is now considered a multicausal disease, with an interplay of acquired and genetic risk factors. Recent studies have shown that patients with the 20210 A prothrombin mutation display remarkably similar characteristics compared with patients with Factor V Leiden mutation. It is evident that neither the Factor V Leiden mutation nor the 20210 A prothrombin mutation is a major risk factor for myocardial infarction or stroke, unless accompanied by other classical risk factors, including diabetes mellitus, hypertension and smoking. Finally, the homozygous form of the thermolabile methylenetetrahydrofolate reductase gene, although leading to elevated homocysteine levels, seems not to represent a genetic risk factor for venous thrombosis. Curr Opin Hematol 1999, 6:291–297

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Abbreviations

APC	activated protein C
MTHFR	methylenetetrahydrofolate reductase

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Venous thrombosis is a frequently occurring disorder with an estimated annual incidence of around one per 1000 people per year. The term "thrombophilia" is used to describe a tendency to develop thrombosis on the basis of inherited or acquired disorders of blood coagulation and fibrinolysis that increase the risk of thrombosis. Examples of acquired risk factors include immobilization, surgery, trauma, plaster casts, lupus anticoagulants, and malignancy. Besides, pregnancy and the use of estrogens have been shown to be associated with venous thrombosis. The entity of thrombophilia was first introduced in 1956 by Jordan and Nandorff [1], who described 43 family members with a thrombotic tendency. In 1965, Egeberg *et al.* [2] reported the first family with antithrombin deficiency and thrombotic tendency. Since then, multiple defects in the genes of anticoagulant proteins have been identified. Until 1994, the prevalence of then-known genetic defects with an established risk of venous thrombosis, *ie*, antithrombin, protein C and protein S, among patients with venous thrombosis was rather low. In a large series of 2132 patients with documented venous thrombosis, the prevalence of one of these deficiencies was 10.9% [3]. In one case-control study [4] in 277 consecutive patients with documented venous thrombosis, the combined prevalence was 8.3%, compared with 2.2% in the 138 control subjects. In a third study [5] in 474 unselected patients, the prevalence was 6.8% versus 3.0% among 474 control subjects. Since 1994, at least four abnormalities have been described that are far more commonly encountered: activated protein C (APC) resistance caused by Factor V Leiden; the 20210 G to A variation in the prothrombin gene (20210 A prothrombin mutation); high levels of Factor VIII; and hyperhomocysteinemia. This article reviews recent studies performed in patients with Factor V Leiden, the 20210 A prothrombin mutation, and hyperhomocysteinemia.

Factor V Leiden

In physiologic circumstances, thrombin, when bound on the endothelial surface, activates protein C into APC. Activated protein C then inactivates the cofactors Factor Va and Factor VIIIa. In 1993, a resistance to this APC was described in patients [6]; it was subsequently shown that in more than 95% of cases, this is caused by a single point mutation in the Factor V molecule (FV R506Q), which is called Factor V Leiden [7,8]. In the general population, the allelic frequency varies from 1% to 8% among whites [9], with very high prevalences occurring in South Sweden (up to 15 percent) and the Middle East. Factor V

is extremely rare to absent in nonwhite people [10]. In patients with venous thrombosis, Factor V Leiden has been found in 20 (unselected patients) up to 50% (selected patients) [11]. The risk for venous thrombosis in heterozygous carriers of the Factor V Leiden has been estimated to be increased four- to eightfold.

In a large, comparative, retrospective cohort study in 723 first- and second-degree relatives of 150 index patients, Martinelli *et al.* [12] found relative risks of 8.1 (95% confidence interval (CI) 3.4 to 19.6) for antithrombin, 7.3 (95% CI 2.9 to 18.4) for protein C, and 8.5 (95% CI 3.5 to 20.8) for protein S deficiency, whereas the risk ratio for Factor V Leiden in this study was 2.2 (95% CI 1.1 to 4.7). The risk of thrombosis for subjects with Factor V Leiden in this study thus seemed lower than the risk for subjects with all three other coagulation defects. However, in the Leiden Thrombophilia Study [13], which had a case-control design, a relative risk of 7 was found for patients with heterozygous Factor V Leiden mutation, versus a relative risk for protein C, protein S, and antithrombin of 6.5, 1.5, and 5.0 respectively.

Whether Factor V Leiden is a risk factor for thrombosis in older patients was investigated in 79 patients with a mean age of 83 years who had had at least one proven episode of venous thromboembolism. The prevalence of Factor V Leiden mutation was 11.4%; only one patient had an antithrombin deficiency [14]. The researchers concluded from this study that even at a higher age, Factor V Leiden is a frequently occurring risk factor.

A new Factor V mutation associated with resistance to protein C (Factor V Cambridge, Arg306→Thr) was found in one patient with venous thrombosis and confirmed APC resistance in the absence of the common R506 Q mutation [15]. Subsequent screening of 585 patients with venous thrombosis, and 226 blood donors, did not reveal any other patient with this mutation, so the authors concluded that the Thr306 mutation is not a common polymorphism but a rare cause of APC resistance.

The 20210 A prothrombin mutation

This prothrombotic mutation involves a guanine-to-adenine transition at nucleotide 20210 in the untranslated region of the gene encoding prothrombin. In a population-based patient control study (the Leiden Thrombophilia Study) [16], heterozygosity for the 20210A allele was found more frequently among consecutive patients with a first deep-vein thrombosis (6.2%) than in the control subjects (2.2%). The relative risk for thrombosis associated with the prothrombin 20210A allele was 2.8 (95% CI 1.4 to 5.6). Heterozygous carriers had significantly higher levels of prothrombin (1.32 U/mL) than noncarriers (1.05 U/mL). Thus, it is likely that the prothrombin 20210A allele acts through the elevated plasma prothrombin levels.

Geographic distribution

In a comparative study [17•], the 20210 G to A prothrombin variant was proved to follow a somewhat different geographic pattern from the Factor V Leiden mutation. After combining data from 11 centers in nine countries, the overall prevalence was calculated. Among 5527 people, 111 heterozygous carriers of the 20210 A allele had been found. The prevalence estimates varied from 0.7 to 4.0 between the centers, and the overall prevalence estimate was 2.0 percent (95% CI 1.4–6.2%). Interestingly, in southern Europe the prevalence was 3.0 percent (95% CI 2.3 to 3.7%), whereas in northern Europe, the prevalence was 1.7% (95% CI 1.3 to 2.2%). The 20210 A allele was very rare in people of Asian and black descent.

Homozygous G20210A prothrombin mutation

In reviewing data from three case studies [18–20], it appears that carriers of a homozygous 20210 A mutation, *ie*, carriers with homozygous Factor V Leiden mutation, do not have as severe thrombotic diathesis as those with homozygous protein C or protein S deficiency (in which purpura fulminans is often encountered) or homozygous type I antithrombin deficiency (which is thought to be incompatible with life) [21]. Most homozygous carriers have experienced early, spontaneous, sometimes severe thrombotic disease. Interestingly, one 72-year-old asymptomatic patient had homozygous 20210 A prothrombin and underwent two episodes of major surgery without prophylactic treatment [20].

Cerebral vein thrombosis

Cerebral vein thrombosis is a rare manifestation of thrombotic disease with a high mortality rate, estimated to be 5% to 30%. It has been found to be associated with Factor V Leiden, the use of oral contraceptives, and pregnancy. The relation between the 20210 A prothrombin gene mutation and cerebral-vein thrombosis, as well as the potential interaction with the use of oral contraceptives, has been studied in 40 unrelated patients with cerebral-vein thrombosis who were compared with 120 healthy control subjects [22•]. The prevalence of the prothrombin mutation was significantly higher in the patients than in the healthy control subjects, 20% and 3% respectively (odds ratio, 10.2; 95% CI 2.3 to 10). The use of oral contraceptives was more frequent in patients (96%) than in control subjects (32%; odds ratio, 22.1; 95% CI 5.9 to 84.2). For women who both had the prothrombin gene mutation and used oral contraception, the odds ratio for cerebral-vein thrombosis was 149.3 (95% CI 31.0 to 711.0). These results are in line with data of another study [23], in which the prevalence of Factor V Leiden was 14% among 36 patients with cere-

bral vein thrombosis, versus an expected prevalence in the general population of 4% to 5%. The age-adjusted odds ratio for venous thrombosis in oral contraceptives users was 13 (95% CI 5 to 37) in this study [23]. In a third study [24], 45 patients with cerebral vein thrombosis were compared with 354 healthy blood donors and 131 patients with either acute ischemic stroke or transient ischemic attack. A total of four patients with cerebral-vein thrombosis (8.9%) had the prothrombin gene mutation, versus eight of 354 (2.3%) in the healthy controls. In contrast, three of 131 (2.3%) subjects (2.3%) with an acute cerebrovascular accident or transient ischmemic attack had the mutation, indicating that the 20210A prothrombin mutation plays only a minor role in arterial ischemic cerebral events.

Risk of recurrent venous thromboembolism

One of the main reasons to investigate both a patient after an episode of venous thrombosis and his family members for inherited disorders of blood coagulation is to give specific therapeutic and prophylactic recommendations on the basis of laboratory findings. Although many articles appear on the prevalence of inherited risk factors and their related relative risk in patients with established venous and arterial thrombosis, few studies exist that have assessed the incidence of recurrent venous thromboembolism after stopping oral anticoagulant treatment in patients with a genetic defect who have had venous thrombosis, because initially, these patients were mostly put on lifelong anticoagulant treatment. Second, no studies have yet assessed the incidence of a first thromboembolic event in asymptomatic but affected family members, nor are studies available that have compared duration or intensity of oral anticoagulant treatment in patients with a history of venous thrombosis and an inherited defect.

Referring to the first type of study, Eichinger *et al.* [25] studied the risk of early recurrent venous thromboembolism in a follow-up study of 492 patients with a history of venous thromboembolism. The 20210 A allele was present in 42 patients (8.5%). Recurrent venous thromboembolism had to be confirmed by objective tests. Duration of anticoagulant therapy had to be at least three months. Mean follow-up time was 26 months, plus or minus 18 months, for the 20210 A positive group, and 25 months, plus or minus 18 months, for the 20210 A negative group. Three patients (7%) with the 20210 A allele underwent a recurrent thromboembolic event, versus 54 patients (12%) without the mutation. The cumulative probability of recurrence did not differ between patients with or without the 20210 A mutation. It was concluded that the risk of early recurrence venous thromboembolism was not higher in heterozygous carriers of the 20210 A mutation than in patients without the mutation. In this study [25], however, the exact duration of oral anticoagulation was not predetermined and varied greatly among the patients. The results of this study are corroborated by a very recent study by Kearon *et al.* [26], who randomly assigned patients with a first episode of idiopathic thromboembolism after three months of oral anticoagulation to either continue warfarin therapy (international normalized ratio 2.0–3.0) or to receive placebo for 24 months. After an average follow-up period of 10 months, the rate of recurrent thromboembolic events among 83 patients who received warfarin for three months was 27.4% per patient-year (17 patients had a recurrence), versus 1.3% per patient-year (one of the 79 patients had a recurrent event) among those who received prolonged oral anticoagulant treatment (95% risk reduction). Three patients (3.8%) in the warfarin group had nonfatal major bleeding, compared with none of those assigned to the placebo group, a statistically significant difference. The prevalence of Factor V Leiden mutation was 26% and the prevalence of the 20210 A prothrombin mutation was 5%. It appeared that in this study [26], these mutations were not associated with recurrent venous thromboembolism. In comparison, researchers in two earlier studies evaluated the risk of recurrent venous thromboembolism in patients with Factor V Leiden. Ridker *et al.* [27] followed 77 patients with idiopathic venous thromboembolism. Seven patients among 63 genetically unaffected people had a recurrence, an incidence of 11.1%, whereas four recurrences developed among 14 heterozygotes for Factor V Leiden, for an incidence of 28.6%. In the second study [28], the recurrence rate of venous thromboembolism among 112 Factor V Leiden patients was 9.9% after a median observation time of 19.3 months, and 9.7% in 268 patients without Factor V Leiden mutation.

Thus, it remains uncertain whether Factor V Leiden or the 20210 A prothrombin mutation confer a greatly increased risk for recurrent venous thromboembolism after a period of oral anticoagulation of 3 to 6 months. A clear need exists for prospective studies wherein patients with thromboembolism and Factor V Leiden or 20210 A prothrombin mutation are followed for a fixed duration of prolonged, then possibly less intensive, anticoagulant treatment. Such studies are now either planned or already started [29].

Factor V Leiden, the prothrombin 20210 A allele, and arterial thrombosis

Although the association between venous thrombosis and Factor V Leiden or 20210 A prothrombin mutation is well demonstrated, most data on the risk of arterial thrombotic events indicate that the carriership of these gene defects does not confer a major risk for arterial disease. In a large study of 560 middle-aged men with a first myocardial infarction before age 70, the prevalence

of the 20210 A variant of the prothrombin gene was 1.8%, whereas in the control group this prevalence was 1.2% [30]. This indicates that the risk of myocardial infarction was increased by 50%, but the 95% confidence interval was wide and not incompatible with the absence of a risk excess (odds ratio 0.6 to 3.8). For carriership of Factor V Leiden, the risk of myocardial infarction was increased by 40%; the 95% confidence limits ranged from 0.8 to 2.2. These data suggest a modest increase in risk for the prothrombin mutation, which was substantially increased if a gene defect coincided with one of the major cardiovascular risk factors like obesity, smoking, hypertension, or diabetes mellitus, with resulting odds ratios varying between 3 and 6. Importantly, in the absence of the coagulation defects, these risks then exceeded those of the single effects. These results confirm two other studies performed in young women ages 18 to 44 years, in whom much higher risks were found (two- to fourfold increase for gene defect carriers) and for whom the relative risk of carriers of either Factor V Leiden or the 20210 A prothrombin allele was especially high if another major cardiovascular risk factor was present (increased 20- to 40-fold) [31,32]. All three studies may be interpreted as examples of interactions between atherosclerotic risk factors and hemostatic defects, which appear to be more relevant in younger patients. This is further underlined by a population based study of 5201 men and women over age 65 who were followed for a mean time of 3.4 years. Among 373 incident cases of myocardial infarction, angina, stroke, or transient ischmemic attack, the Factor V Leiden mutation was not a risk factor for any of these events [33]. Finally, to assess whether either Factor V Leiden mutation or the 20210 A prothrombin allele increase the risk of stroke in young women, in a case-control study [34], 106 women ages 18 to 44 years who all had undergone a first stroke were compared with 391 control subjects who had not undergone stroke. It was concluded that for young women who had undergone stroke, neither Factor V Leiden nor the 20210 A prothrombin mutation is an important risk factor.

Combined 20210 A prothrombin mutation and Factor V Leiden mutation

Since Factor V Leiden has been discovered as the most common inherited defect of blood coagulation, it has become apparent that venous thrombosis often should be considered as being caused by multiple factors. Many inherited and acquired risk factors could cosegregate with Factor V Leiden, and this could possibly lead to thrombosis both becoming manifest in younger patients and having increased severity. For protein C, protein S, and antithrombin, a higher risk of thrombosis has been demonstrated in three studies [35–37].

To assess how often the 20210 A prothrombin mutation is co-inherited with Factor V Leiden mutation, Ehrenfort *et al.* [38] studied 200 carriers of Factor V mutation, 150 of whom had had a thrombosis before age 45, and 200 healthy control subjects, in all of whom FII genotyping was performed. The 20210 A allele was found in four of the 200 healthy controls, a prevalence of 2%. Among the 50 asymptomatic people with Factor V Leiden, the prevalence was 4%. In contrast, of the 115 patients with heterozygous Factor V Leiden mutation, 14 (12.2%) had the 20210A allele, whereas five of the 35 patients (14%) with homozygous Factor V Leiden also had a 20210 A allele. In the presence of the 20210 A allele, the risk for thrombosis occurring at a young age was increased threefold in patients with heterozygosity for Factor V Leiden, which itself was shown to increase the risk for thrombosis around fourfold. The median age at which patients underwent a first thrombosis was 27 years for those with double defects, 33 years for those with the 20210 A allele, and 29 years for those with Factor V Leiden. In another study [39] of 332 people from 53 families with APC resistance, both the 20210 A prothrombin and Factor V Leiden mutations were tested. Of 29 persons with Factor V Leiden mutation, three (10%) also had the 20210 A prothrombin mutation, which was higher than the 1.8% (five of 282) found in the healthy control subjects. Both reports differ from a large study by Alhenc-Gelas *et al.* [40], who did not find any 20210 A prothrombin mutation among 151 subjects with heterozygous Factor V Leiden mutation. An explanation of the disparate findings might be that in the latter study [40], most subjects (66 of 151) were asymptomatic and possibly older than the subjects in the former two studies. It is concluded that, like Factor V Leiden, the 20210 A prothrombin mutation may increase the risk of thrombosis in people affected with protein C, protein S, or antithrombin deficiency.

Hyperhomocysteinemia

Mild hyperhomocysteinemia has been shown to be a risk factor for venous thrombosis and may be caused by genetic and environmental factors. Den Heijer *et al.* [41•] performed a meta-analysis of ten case-control studies on venous thrombosis and found a pooled estimate of the odds ratio of 2.5 (95% CI 1.8–3.5) for a fasting homocysteine level above the 95th percentile. The most common genetic variant that results in hyperhomocysteinemia is caused by a substitution in methylenetetrahydrofolate reductase (MTHFR), the enzyme that converts 5,10-methylenetetra-hydrofolate to 5-methyltetra-hydrofolate, the carbon donor in homocysteine remethylation by methionine synthase [42]. The resultant 677T variant is thermolabile and has reduced activity in people who are homozygous or heterozygous for this mutation. Debate has occurred as to whether this variant is a risk factor for thrombosis. Initial reports

showed this variant to be a risk factor, especially in combination with Factor V Leiden mutation, whereas other reports did not support the hypothesis. In three reports, investigators have studied the risk for venous thrombosis of the 677T variant either with or without the Factor V Leiden mutation or the 20210 A prothrombin mutation. Kluijtmans *et al.* [43] studied the frequency of the homozygous 677C → T mutant genotype of the *MTHFR* gene in 471 patients with deep-vein thrombosis and 474 healthy controls and its interaction with Factor V Leiden mutation. Homozygosity for the 677T variant was observed in 10% of the patients and 9.9% of the control subjects. In addition, no modified risk of the genotype was observed in carriers of Factor V Leiden. In the second study [44], using a somehow similar approach but with the 20210 A prothrombin mutation as a second variable, 558 unselected patients with venous thromboembolism were compared with 500 control subjects. The odds ratio for the MTHFR C677T variant heterozygosity and homozygosity were 1.07 (95% CI 0.84 to 1.36) and 0.71 (95% CI 0.48 to 1.03), respectively. In patients with the Factor V Leiden or 20210 A prothrombin mutation, no increase in risk caused by the MTHFR 677T variant was seen. In the third study, Margaglione *et al.* [45] studied 277 consecutive patients with confirmed deep-vein thrombosis and 431 healthy subjects. The homozygous 677T variant was more frequent in patients than in control subjects (25.6% versus 18.1%, respectively; $P = 0.016$). The risk of thrombosis among carriers of this genotype was significantly increased (odds ratio 1.6; 95% CI 1.1 to 2.3). The estimated risk associated with the homozygous 677T variant was 2.0 (95% CI 1.3 to 3.1) for those patients with predisposing risk factors, including Factor V Leiden, prothrombin mutation, or acquired risk factors, but the estimated risk stayed significant at 1.7 (95% CI 1.2 to 2.6) after adjustment for gender, Factor V Leiden, and 20210 A prothrombin mutation. These authors [45] concluded, in contrast to the previous two studies, that the homozygous MTHFR 677T genotype is an independent risk factor for venous thrombosis.

Several conclusions related to homocysteinemia and venous thrombosis can be drawn. First, the relation between mild homocysteinemia and venous thrombosis has been clearly established. Second, most studies have not found an association of homozygosity of the thermolabile MTHFR and increased risk for venous thrombosis. Third, published studies on the interaction between Factor V Leiden or the 20210 A prothrombin mutation and the thermolabile MTHFR are controversial. Third, although in one study a clear interaction between hyperhomocysteinemia an Factor V Leiden was observed (when compared with men with neither abnormality, those affected by both disorders had a 10 fold increase in risk of venous thromboembolism) [46] in another study the combined effect was smaller than for Factor V Leiden alone [47] and it is thus yet unclear whether such an interaction really exists. Finally, clinicians must realize that intervention studies with pharmacological lowering of homocysteine levels are currently be performed and should give definitive answers on the relevance of homocysteine lowering towards reduction of thrombotic risk.

Conclusions

Thrombophilia can now be considered a multicausal disease in which genetic and acquired risk factors interact with each other [48]. Among the common genetic abnormalities of coagulation, numerous studies have been performed in the previous year in patients with the recently discovered 20210 A prothrombin mutation. From these studies, the concept has emerged that the clinical features of patients with a 20210 A prothrombin mutation are remarkably comparable with those of patients with Factor V Leiden mutation. In spite of a slight shift towards the southern European population, the 20210 A prothrombin mutation is prevalent in the white population and very rare in people of black and Asian descent. The homozygous prothrombin mutation does not lead to the severe thrombotic disease as does protein C deficiency or antithrombin deficiency, although most homozygous carriers experience early and sometimes severe thrombotic episodes. It is uncertain whether the 20210 A prothrombin mutation confers a risk for increased recurrent venous thromboembolism. As for Factor V Leiden, no major role seems to exist for the prothrombin mutation in relation to arterial thrombotic complications, unless patients have other, more classical risk factors for arterial ischemic disease. Finally, if a combination of Factor V Leiden and 20210 A prothrombin mutation occurs, it may lead to higher risk for thrombosis. With respect to hyperhomocysteinemia, it is now evident that although homozygosity for the 677T polymorphism of MTHFR is associated with increased homocysteine levels, this homozygous mutation by itself is not an independent risk factor for deep vein thrombosis, regardless of the Factor V Leiden genotype.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as

- • Of special interest
- •• Of outstanding interest

1 Jordan FLJ, Nandorff A The familial tendency in thromboembolic disease. Acta Med Scand 1956, 156:267–275

2 Egeberg O Inherited antithrombin deficiency causing thrombophilia. Thromb Diathesis Haemorrhagica 1965, 13:516–530

3 Mateo J, Oliver A, Borrell M, Sala N, Fontcuberta J Laboratory evaluation and clinical characteristics of 2132 consecutive unselected patients with venous thromboembolism: results of the Spanish multicentric study on thrombophilia Thromb Haemost 1997, 77:444–451

4 Heyboer H, Brandjes DPM, Bueller HR, Sturk A, ten Cate JW Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis N Engl J Med 1990, 323:1512–1516

5 Koster T, Rosendaal FR, Briet E, van der Meer FJM, Colly LP, Trienekens PH, et al Protein C deficiency in a controlled series of unselected outpatients an infrequent but clear risk factor for venous thrombosis Blood 1995, 85 2756–2761

6 Dahlbaeck B, Carlsson M, Svenson PJ Familial thrombophilia due to a previously unrecognised mechanism characterised by poor anticoagulation response to activated protein C prediction of a cofactor to activated protein C Proc Natl Acad Sci U S A 1993, 90 1004–1008

7 Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven R, de Ronde H et al Mutation in blood coagulation Factor V associated with resistance to activated protein C Nature 1994, 369 64–67

8 Zoller B, Svensson P, He X, Dahlback B Identification of the same Factor V gene mutation in 47 out of 50 thrombosis prone families with inherited resistance to activated protein C J Clin Invest 1994, 94 2521–2524

9 Rees DC, Cox M, Clegg JB World distribution of Factor V Leiden Lancet 1995, 346 1133–1134

10 De Stefano V, Chiusolo P, Paciaroni K, Leone G Epidemiology of Factor V Leiden clinical implications Semin Thromb Hemost 1998, 24 367–379

11 Koster T, Rosendaal FRR, de Ronde H Briet E Venous thrombosis due to poor anticoagulant response to activated protein C Leiden Thrombophilia Study Lancet 1993, 342 1503–1506

12 Martinelli I, Mannucci PM, De Stefano V, Taioli E, Rossi V, Crosti F, et al Different risks of thrombosis in four coagulation defects associated with inherited thrombophilias a study in 150 families Blood 1998 92 2353–2358

13 van der Meer FJM, Koster T, Vandenbroucke JP Briet E, Rosendaal FR The Leiden Thrombophilia Study (LETS) Thromb Haemost 1997, 78 631–635

14 Andre E Siguret V, Alhenc Gelas M, Saint Jean O, Gaussem P Venous thrombosis in older people prevalence of the factor v gene mutation q506 J Am Geriatr Soc 1998, 46 1545–1549

15 Williamson D, Brown K, Luddington R, Baglin C Baglin T Factor V Cambridge a new mutation (Arg 306 → Thr) associated with resistance to activated protein C Blood 1998, 91 1140–1144

16 Poort SR, Rosendaal FR, Reitsma PH, Bertina RM 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 1996, 88 3698–3703

17 • Rosendaal FR Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, et al Geographic distribution of the 20210 G to A prothrombin variant Thromb Haemost 1998, 79 706–708

This article is relevant when assessing etiology of a thrombosis, because parallel to Factor V Leiden, the 20210A prothrombin mutation is very rare to absent in nonwhite people

18 Kyrle PA, Mannhalter C, Beguin S, Stumpflen A, Hirschl M, Weltermann A, et al Clinical studies and thrombin generation in patients homozygous or heterozygous for the G20210A mutation in the prothrombin gene Arterioscler Thromb Vasc Biol 1998, 18 1287–1291

19 Zawadzki C Gaveriaux V, Trillot N, Bauters A Watel A, Alhenc Gelas M, et al Homozygous G20210A transition in the prothrombin gene associated with severe venous thrombotic disease two cases in a French family Thromb Haemost 1998, 80 1027–1028

20 Alatri A, Franchi F, Moia M Homozygous G20210A prothrombin gene mutation without thromboembolic events a case report Thromb Haemost 1998 80 1028–1029

21 Lane DA, Mannucci PM, Bauer KA Bertina RM, Bochkov NP, Boulyjenkov V, et al Inherited thrombophilia part 2 Thromb Haemost 1996, 76 824–834

22 • Martinelli I, Sacchi E, Landi G, Taioli G, Duca F Mannucci PM High risk of cerebral-vein thrombosis in carriers of a prothrombin gene mutation and in users of oral contraceptives N Engl J Med 1998, 338 1793–1397

In this article, the authors support evidence of earlier observations that have demonstrated an interaction between gene (Factor V Leiden of 20210A prothrombin mutation) and environment (oral contraceptives)

23 de Bruijn SFTM, Stam J, Koopman MMW, Vandenbroucke JP Case control study of risk of cerebral sinus thrombosis in oral contraceptive users who are carriers of hereditary prothrombotic conditions BMJ 1998, 316 589–592

24 Reuner KH Grau A Rickmann H, Stolz E, Juttler E, Druschky KF, Patscheke H Prothrombin gene G20210 → A transition is a risk factor for cerebral venous thrombosis Stroke 1998, 29 1765–1769

25 Eichinger S Minar E, Hirschl M Bialonczyk C Stain M, Mannhalter C, et al The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene Thromb Haemost 1999, 81 14–17

26 Kearon C, Gent M, Hirsh J, Weitz J, Kovacs MJ, Anderson DR, et al A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism N Engl J Med 1999, 340 901–907

27 Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH Factor V Leiden and risks of recurrent idiopathic venous thromboembolism Circulation 1997, 92 2800–2802

28 Eichinger S, Pabinger I, Stumpflen A, Hirschl M, Bialonczyk C, Schneider B, et al The risk of recurrent venous thromboembolism in patients with and without Factor V Leiden Thromb Haemost 1997, 77 624–628

29 Ridker PM Long-term, low dose warfarin among venous thrombosis patients with and without Factor V Leiden mutation rationale and design for the prevention of recurrent venous thromboembolism (PREVEN˜) trial Vasc Med 1998, 3 67–73

30 Doggen CJM, Manger Cats V, Bertina RM, Rosendaal FR Interaction of coagulation defects and cardiovascular risk factors Increased risk of myocardial infarction associated with Factor V Leiden or prothrombin 20210A Circulation 1998, 97 1037–1041

31 Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL A common prothrombin variant (20210A) increases the risk of myocardial infarction in young women Blood 1997, 90 1747–1750

32 Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL Factor V Leiden (resistance to protein C) increases the risk of myocardial infarction in young women Blood 1997, 89 2817–2821

33 Cushman M, Rosendaal FR, Psaty BM, Cook EF, Vailliere J, Kuller LH, Tracy RP Factor V Leiden is not a risk factor for arterial vascular disease in the elderly results from the cardiovascular health study Thromb Haemost 1998 79 912–915

34 Longstreth WT Jr, Rosendaal FR, Siscovick DS, Vos HL, Schwartz SM, Psaty BM, et al Risk of stroke in young women and two prothrombotic mutations Factor V Leiden and prothrombin gene variant (G20210A) Stroke 1998, 29 577–580

35 Koeleman BPC, Reitsma P, Allaart CF, Bertina RM APC resistance as an additional risk factor for thrombosis in protein C-deficient families Blood 1994 84 1031–1035

36 Zoller B Berntsdotter A, Garcia de Frutos P, Dahlback B Resistance to activated protein C as an additional factor for thrombosis in protein C deficient families Blood 1995, 85 3518–3523

37 Van Boven HH Reitsma P Rosendaal FR, Bayston TA, Chowdhury V, Bauer KA, et al Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency Thromb Haemost 1996, 75 417–421

38 Ehrenfort S, Ludwig G, Klinke S, Krause M, Scharrer I, Nowak Goettl U The prothrombin 20210 A allele is frequently combined in young carriers of the Factor V Arg 506 to Gln mutation with venous thrombophilia Blood 1998, 91 2209–2210

39 Zoller B, Svensson PJ, Dahlback B, Hillarp A The A20210 allele of the prothrombin gene is frequently associated with the Factor V Arg 506 to Gln mutation but not with protein S deficiency in thrombophilic families Blood 1998, 91 2210–2211

40 Alhenc Gelas M, Le Cam-Duchez V, Emmerich J, Frebourg T, Fiessinger Jn, Borg JY, Aiach M The A20210 allele of the prothrombin gene is not frequently associated with the Factor V Arg 506 to Gln mutation in thrombophilic patients Blood 1997, 90 1711

41 • Den Heijer M, Rosendaal FR Blom HJ, Gerrits WBJ, Bos GMJ Hyperhomocysteinemia and venous thrombosis a meta analysis Thromb Haemost 1998, 80 874–877

In this article, the authors give a nice state of-the art overview of homocysteinemia and venous thrombosis during 1998

42 Frosst P, Blom H, Milos R, Goyette P, Sheppard CA, Matthews RG, et al A candidate genetic risk factor for vascular disease a common mutation in methylenetetrahydrofolate reductase Nature Genet 1995, 10 111–113

43 Kluijtmans LAJ, den Heijer M, Reitsma PH, Heil S, Blom HJ, Rosendaal FR Thermolabile methylenetetrahydrofolate reductase and Factor V Leiden in the risk of deep vein thrombosis Thromb Haemost 1998, 79 254–258

44 Brown K, Luddington R Baglin T Effect of the MTHFRC677T variant on risk of venous thromboembolism interaction with Factor V Leiden and prothrombin (F2G20210A) mutations Br J Haematol 1998, 103 42–44

45 Margaglione M D'Andrea G, d'Addedda M, Giuliani N Cappucci G, Iannaccone L, et al The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independently of the coexis-

tence of the FV Leiden and the prothrombin 20210A mutation. Thromb Haemost 1998, 79:907–911.

46 Ridker PM, Hennekens Ch, Selhub J, Miletich JP, Malinow R, Stampfer M: Interrelation of hyperhomocysteinemia, Factor V Leiden and the risk of future venous thromboembolism. Circulation 1997, 95:1777–1782.

47 Den Heijer M, Koster T, Blom HJ, Bos GMJ, Briet E, Reitsma PH, et al.: Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 1996, 334:759–762.

48 Rosendaal FR: Venous thrombosis: a multicausal disease. Lancet 1999, 353:1176–1173.