CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Ethnic differences in the prevalence of inherited thrombophilic polymorphisms in an asymptomatic Australian prenatal population
Authors	Said, Joanne M.;Brennecke, Shaun P.;Moses, Eric K.;Walker, Susan P.;Borg, Anthony J.;Williams, Jeff T.;Higgins, John R.
Publication date	2006-08-01
Original Citation	Said JM; Brennecke SP; Moses EK; Walker SP; Borg AJ; Williams JT; Higgins JR; (2006) 'Ethnic differences in the prevalence of inherited thrombophilic polymorphisms in an asymptomatic Australian prenatal population'. Human biology; an international record of research, 78 (4):403-412.
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://muse.jhu.edu/journals/human_biology/v078/78.4said.html - 10.1353/hub.2006.0058
Rights	Copyright © 2006 by The Wayne State University Press. All rights reserved.
Download date	2026-11-06 16:44:12
Item downloaded from	https://hdl.handle.net/10468/480

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Ethnic Differences in the Prevalence of Inherited Thrombophilic Polymorphisms in an Asymptomatic Australian Prenatal Population

JOANNE M. SAID,[1] SHAUN P. BRENNECKE,[2] ERIC K. MOSES,[3] SUSAN P. WALKER,[4] ANTHONY J. BORG,[3] JEFF T. WILLIAMS,[5] AND JOHN R. HIGGINS[6]

Abstract Differences in the prevalence of thrombophilias in different ethnic populations have been demonstrated. Because the Australian population includes many different ethnic groups, we sought to assess the effect of ethnicity in our Australian prenatal population on the prevalence of thrombophilic polymorphisms. Asymptomatic, nulliparous women ($n = 1{,}129$) recruited for a large prospective study were included in this analysis. These women had no personal or family history of venous thromboembolism and were not known to be carrying an inherited or acquired thrombophilia. Ethnicity was determined at recruitment, and women were categorized as being of Northern European, Southern European, Middle Eastern, Asian, or Other ethnicity. These women underwent genotyping for the following polymorphisms: factor V Leiden G1691A, prothrombin gene A20210G mutation, methylenetetrahydrofolate reductase (*MTHFR*) C677T and A1298C, and thrombomodulin C1418T. The factor V Leiden allele was seen significantly more frequently in patients of Middle Eastern background compared to those of Northern European and Asian ethnicity ($p < 0.05$). The prothrombin gene mutation was seen significantly more frequently in patients of Southern European ethnicity compared to those of Northern European or Asian ethnicity ($p < 0.05$). The *MTHFR* C677T allele (mutant) was significantly less common in those of Asian ethnicity compared to patients of Northern European

[1]Fellow in Maternal Fetal Medicine, Royal Women's Hospital; and Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia.

[2]Royal Women's Hospital Department of Obstetrics and Gynaecology, University of Melbourne; and Department of Perinatal Medicine, Royal Women's Hospital, Melbourne, Australia.

[3]Department of Perinatal Medicine, Royal Women's Hospital, Melbourne, Australia. Current address: Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

[4]Department of Obstetrics and Gynaecology, University of Melbourne; and Mercy Hospital for Women, Melbourne, Australia.

[5]Department of Genetics, Southwest Foundation for Biomedical Research; and Southwest National Primate Research Center, San Antonio, Texas.

[6]Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia. Current address: Department of Obstetrics and Gynaecology, University College, Cork, Ireland.

Human Biology, August 2006, v. 78, no. 4, pp. 403–412.
Copyright © 2006 Wayne State University Press, Detroit, Michigan 48201-1309

KEY WORDS: THROMBOPHILIA, FACTOR V LEIDEN, PROTHROMBIN GENE, METHYLENETETRAHYDROFOLATE REDUCTASE (*MTHFR*), THROMBOMODULIN, AUSTRALIA, PRENATAL POPULATION.

and Southern European ethnicity ($p < 0.0005$). There were no significant differences seen with the *MTHFR* A1298C polymorphism. The mutant thrombomodulin allele was seen significantly more frequently in Asian women compared to Northern European, Southern European, or Middle Eastern women ($p < 0.005$). There are important ethnic differences in the prevalence of thrombophilic polymorphisms in the Australian prenatal population.

Australia is a melting pot of diverse ethnicities. As a relatively new country, Australia has a history of different migration patterns, ranging from the early days of Anglo-Celtic colonization to the more recent patterns of Southern European, Southeast Asian, African, and Middle Eastern migration. Several studies have already demonstrated significant differences in the prevalence of a number of genetic thrombophilias (Rees et al. 1995; Herrmann et al. 1997; Gudnason et al. 1998; Rosendaal et al. 1998), but there are limited data for the Australian population.

Inherited thrombophilias predispose carriers to venous thromboembolism (Gerhardt et al. 2000), yet most of the carriers remain asymptomatic. Recent associations between thrombophilias and adverse pregnancy outcomes (Alfirevic et al. 2002; Rey et al. 2003; Said and Dekker 2003) have prompted calls for screening and institution of anticoagulant therapy in high-risk populations (Bates et al. 2004). To fully consider the merits of such propositions, we need a more detailed assessment of the prevalence of these conditions in prenatal populations. Furthermore, not all studies have confirmed an association between adverse pregnancy outcomes and inherited thrombophilias (Livingston et al. 2001; Infante-Rivard et al. 2002). It is possible that differences in the prevalence of inherited thrombophilias in different ethnic groups may contribute to apparent contradictions between studies.

We sought to assess the effect of ethnicity on the prevalence of five common thrombophilic polymorphisms in our Australian prenatal population: factor V Leiden polymorphism, which has been well described as the most common thrombophilia predisposing carriers to venous thrombosis in Caucasian populations (Dahlback 1997); the prothrombin gene mutation A20210G, which is associated with elevated circulating prothrombin levels (Poort et al. 1996); two polymorphisms (C677T and A1298C) in the methylenetetrahydrofolate reductase gene (*MTHFR*), which contribute to the development of hyperhomocysteinemia (Frosst et al. 1995; van der Put et al. 1998); and a common polymorphism in the thrombomodulin gene (C1418T), which interferes with thrombin binding on the endothelial surface and results in impaired activation of the naturally occurring anticoagulant protein C (Dittman and Majeurs 1990).

Methods

Nulliparous women were recruited for this study from the prenatal clinics at the Royal Women's Hospital and the Mercy Hospital for Women, in Melbourne,

Australia, as part of a study designed to assess the overall prevalence of thrombophilias in asymptomatic women and evaluate the risk of adverse pregnancy outcomes in thrombophilic women. Patients were recruited before they reached 22 weeks' gestation, and written consent for participation in the study was obtained. Ethnicity was determined at the time of recruitment by asking a series of questions about the patient's ethnicity as well as that of their parents, grandparents, and ancestors. This project was approved by the Institutional Ethics Committees at both hospitals. To obtain a truly asymptomatic population, which would not otherwise be suspected of carrying a thrombophilia, women were excluded from recruitment if they were known to be carrying a thrombophilia, had a family history of thrombophilia, had a past history or family history of venous thromboembolism, or had more than two previous miscarriages or a previous midtrimester miscarriage.

Because of the detailed nature of the consent process required, it was not possible to recruit non-English-speaking women.

Venous blood was obtained from the participants in vials containing EDTA, and a buffy coat layer was prepared from whole blood. Genomic DNA was then extracted from peripheral blood leukocytes using a commercially available kit (Puregene Whole Blood Kit, Gentra Systems, Minneapolis, Minnesota). Genotyping for the five polymorphisms was performed using Taqman assays in an ABI Prism 7700 Sequencer, using primers and fluorescent MGB probes designed using Primer Express Software and validated in the Pregnancy Research Centre at the Royal Women's Hospital. (Primer and probe sequences are given in Table 1.) Each PCR reaction was performed in a 10-μl volume using 2 μl genomic DNA (concentration 10 ng/μl), 5 μl Thermo-Start Q-PCR MasterMix (ABgene, Applied Biosystems, Foster City, California), 0.09 μl of the forward and reverse primers, 0.02 μl FAM and VIC labeled fluorescent probes, 0.03 μl Rox, and 2.75 μl H_2O, with the following PCR thermocycler conditions: initial activation step at 95°C followed by PCR amplification of 40 cycles (denaturation at 95°C for 15 s, primer annealing at 62°C, extension at 72°C for 30 s) and a final extension step at 72°C for 5 min. Fluorescence detection of PCR products was carried out in the 7700 Sequence Detector according to the manufacturer's recommendations.

Statistical Analysis. The prevalence and 95% confidence interval of each polymorphism was derived using Stata 7.0.

Differences between the prevalence in each ethnic subgroup were analyzed using the two-sample test of proportion for independent proportions. A p value less than 0.05 was regarded as significant, indicating that the difference in prevalence between the two ethnic groups was significant.

Results

This study included 1,129 women of known ethnicity. These women had all five polymorphisms successfully genotyped.

Table 1. Primer and Probe Sequences

Polymorphism	*Sequence*
Factor V Leiden	
Forward primer	AGACATCGCCTCTGGGCTAA
Reverse primer	CTGAAAGGTTACTTCAAGGACAAAATAC
FAM probe (wild type)	6FAM-TATTCCTCGCCTGTCC-MGBNFQ
VIC probe (factor V Leiden)	VIC-TGTATTCCTTGCCTGTC-MGBNFQ
Prothrombin gene mutation	
Forward primer	AACCAATCCCGTGAAAGAATTATTT
Reverse primer	CCAGAGAGCTGCCCATGAAT
FAM probe (wild type)	6FAM-TTGAGGCTCGCTGAG-MGBNFQ
VIC probe (prothrombin)	VIC-TTGAGGCTTGCTGAGA-MGBNFQ
MTHFR C677T	
Forward primer	TGGCAGGTTACCCCAAAGG
Reverse primer	CACAAAGCGGAAGAATGTGTCA
FAM probe (C allele)	6FAM-AAATCGGCTCCCGCA-MGBNFQ
VIC probe (T allele)	VIC-ATGATGAAATCGACTCC-MGBNFQ
MTHFR A1298C	
Forward primer	AAGAGCAAGTCCCCCAAGGA
Reverse primer	ACTTTGTGACCATTCCGGTTTG
FAM probe (A allele)	6FAMAAGACACTTTCTTCACTGMGBNFQ
VIC probe (C allele)	VIC-AAGACACTTGCTTCAC-MGBNFQ
Thrombomodulin	
Forward primer	CCTCCCCGGTACCTTCGA
Reverse primer	TCCACCTTGCCGGAGTCA
FAM probe (wild type)	6FAM-CCCTTGCCCGCCAC-MGBNFQ
VIC probe (mutant allele)	VIC-CCCTTGTCCGCCACA-MGBNFQ

Most of the women were of Northern European descent (64%). Southern European and Asian women represented 15% and 13% of the population, respectively, and 6% of the women were from Middle Eastern countries (Lebanon, Israel, and Turkey predominantly). A further 2% of women (25 women) had other ethnic backgrounds, including African ($n = 15$) and Australian Aboriginal ($n = 1$), or were of mixed ethnicity ($n = 9$). Because this last group represented an extremely heterogeneous group and because the numbers in each individual group were too small to provide meaningful results, they were excluded from further analysis, leaving 1,104 women in this study.

The allele frequency of the mutant genes in each ethnic subgroup is shown in Table 2.

Factor V Leiden. We identified 60 women as heterozygous carriers of factor V Leiden. No women were homozygous for this mutation. The allele frequency for each ethnic group is shown in Table 2. The factor V Leiden mutation was least common in patients of Asian background compared to all other ethnic groups ($p < 0.05$). Middle Eastern women were more likely to carry this mutation than

Table 2. Frequency of Mutant Alleles in Different Ethnic Groups

	Northern European ($n = 732$)		*Southern European* ($n = 165$)		*Asian* ($n = 143$)		*Middle Eastern* ($n = 64$)		
Allele	*Number of Mutant Alleles*	*Prevalence (95% CI)*	*Number of Mutant Alleles*	*Prevalence (95% CI)*	*Number of Mutant Alleles*	*Prevalence (95% CI)*	*Number of Mutant Alleles*	*Prevalence (95% CI)*	*Significance of Comparison Between Two Ethnic Groups*[a]
Factor V Leiden	42	2.86% (2.07–3.85)	9	2.73% (1.25–5.11)	1	0.35% (0–1.93)	8	6.25% (2.74–11.94)	NE:SE, $p = 0.89$ NE:A, $p = 0.01$[b] NE:ME, $p = 0.04$[b] SE:A, $p = 0.02$[b] SE:ME, $p = 0.07$ A:ME, $p = 0.0001$[b]
Prothrombin gene mutation	14	0.96% (0.52–1.60)	8	2.42% (1.05–4.72)	1	0.35% (0–1.93)	2	1.56% (0.29–5.53)	NE:SE, $p = 0.01$[b] NE:A, $p = 0.31$ NE:ME, $p = 0.51$ SE:A, $p = 0.02$[b] SE:ME, $p = 0.47$ A:ME, $p = 0.18$
MTHFR C677T	503	34.36% (31.92–36.85)	129	39.09% (33.79–44.58)	61	21.32% (16.73–26.54)	42	32.81% (24.78–41.67)	NE:SE, $p = 0.10$ NE:A, $p < 0.0001$[b] NE:ME, $p = 0.72$ SE:A, $p < 0.0001$[b] SE:ME, $p = 0.21$ A:ME, $p = 0.01$[b]

Table 2. (Continued)

Allele	*Northern European* ($n = 732$)		*Southern European* ($n = 165$)		*Asian* ($n = 143$)		*Middle Eastern* ($n = 64$)		*Significance of Comparison Between Two Ethnic Groups*[a]
	Number of Mutant Alleles	*Prevalence (95% CI)*	*Number of Mutant Alleles*	*Prevalence (95% CI)*	*Number of Mutant Alleles*	*Prevalence (95% CI)*	*Number of Mutant Alleles*	*Prevalence (95% CI)*	
MTHFR A1298C	443	30.26% (27.91–32.68)	105	31.81% (26.82–37.14)	78	27.27% (22.19–32.82)	47	36.71% (28.38–45.69)	NE:SE, $p = 0.58$ NE:A, $p = 0.31$ NE:ME, $p = 0.13$ SE:A, $p = 0.22$ SE:ME, $p = 0.32$ A:ME, $p = 0.05$
Thrombomodulin polymorphism	275	18.78% (16.81–20.88)	40	12.12% (8.80–16.13%)	76	26.57% (21.54–32.09)	8	6.25% (2.74–11.94)	NE:SE, $p = 0.004$[b] NE:A, $p = 0.003$[b] NE:ME, $p < 0.0005$[b] SE:A, $p < 0.0001$[b] SE:ME, $p = 0.07$ A:ME, $p < 0.0001$[b]

a. NE, Northern European; SE, Southern European; A, Asian; ME, Middle Eastern.
b. Significant frequency of the mutant allele between the two ethnic groups indicated.

Northern European women ($p < 0.05$) or Asian women ($p < 0.0005$). No significant differences were seen between Northern and Southern European women.

Prothrombin Gene Mutation. Twenty-five of the 1,104 women were heterozygous carriers of the prothrombin gene mutation. This mutant allele was significantly more common in Southern European women than in Northern European or Asian women ($p < 0.05$). No statistically significant differences were observed in the frequency of the mutant allele in the Middle Eastern population compared to any other ethnic groups.

***MTHFR* C677T.** One hundred thirty-one women were homozygous for the *MTHFR* C677T polymorphism, which is associated with hyperhomocysteinemia. The mutant allele was seen significantly less frequently in Asian women compared to Northern European and Southern European women ($p < 0.0001$) and Middle Eastern women ($p < 0.05$).

***MTHFR* A1298C.** Homozygosity for the *MTHFR* A1298C polymorphism was seen in 111 women, and 451 women were heterozygous for this polymorphism. No statistically significant differences were seen among any of the ethnic groups.

Significant linkage disequilibrium was observed between the two *MTHFR* loci within each ethnic subgroup.

Thrombomodulin Polymorphism. Thirty-eight women were homozygous for the thrombomodulin polymorphism. The mutant allele was significantly more common in Asian women than in all other ethnic groups ($p < 0.005$). In addition, significant differences in the frequency of the mutant allele were also seen between Northern and Southern European women and between Northern European and Middle Eastern women ($p < 0.005$).

All five polymorphisms were in Hardy-Weinberg equilibrium within each ethnic subgroup.

Discussion

These data confirm the findings of previous studies, which have suggested a high prevalence of the factor V Leiden mutation in Middle Eastern subjects (Awidi et al. 1999; Irani-Hakime et al. 2000) and a virtual absence of this polymorphism in Asian populations (Rees et al. 1995; Angchaisuksiri et al. 2000). The prevalence of this polymorphism in our Northern European population is similar to that seen in other Caucasian populations (Lee et al. 1996; Ridker et al. 1997; Livingstone et al. 2000). These differences in the prevalence support the theory that the Factor V Leiden mutation arose in a single common ancestor 21,000 to 34,000 years ago (after the separation of Africans and Asians from Caucasian populations) (Zivelin et al. 1997).

Likewise, the prothrombin gene mutation is rarely seen in Asian populations (Angchaisuksiri et al. 2000; Chan et al. 2000) but is slightly more prevalent in Southern European populations than in Northern European populations (Rosendaal et al. 1998).

The *MTHFR* C677T polymorphism is more prevalent in Caucasian populations than in Asian (Esfahani et al. 2003) or African populations (Conroy et al. 2000; Esfahani et al. 2003). In contrast to the findings of Esfahani et al. (2003), we did not find any significant differences in the prevalence of the *MTHFR* A1298C polymorphism among the different ethnic groups. Significant linkage disequilibrium was observed between the two *MTHFR* loci among all four ethnic groups. This finding is consistent with the previous study by van der Put et al. (1998) but contrasts with the findings of Isotalo et al. (2000), who described the presence of triple and quadruple mutations in fetal samples, which could arise only if linkage disequilibrium were incomplete.

The significantly higher prevalence of the thrombomodulin polymorphism in Asian women is an important finding and requires further investigation of the possible role of this polymorphism in contributing to both venous thromboembolism and adverse pregnancy outcomes in these populations. To our knowledge, this is the largest population in whom the prevalence of this polymorphism has been examined.

We aimed to assess the prevalence of these thrombophilic polymorphisms in a population that would not otherwise be suspected of carrying thrombophilias. As such, we excluded women who had a personal or family history of thromboembolism, women who were known to carry or to have a family history of thrombophilia, and women who had had more than two previous miscarriages or a previous midtrimester miscarriage. There is no doubt that, according to these strict criteria, we will be underestimating the true population prevalence of thrombophilia—this is better achieved using random population-based samples (Gibson et al. 2005). Nevertheless, we have demonstrated that inherited thrombophilias are indeed prevalent in an asymptomatic prenatal population.

As illustrated in Table 2, we performed 30 separate statistical analyses. We acknowledge that this type of multiple analysis may result in the detection of a statistical difference simply by chance alone. Using a Bonferroni adjustment, statistical comparisons resulting in $p < 0.0017$ would still be regarded as significant. Table 2 highlights a number of highly significant differences in the prevalence of thrombophilias between ethnic groups. Significance for a number of these analyses is retained even when the Bonferroni adjustment is applied.

The prevalence of a particular thrombophilia will have a direct effect on the sample size required to study the association between inherited thrombophilias and adverse pregnancy outcomes. As this study and previous studies have demonstrated, the prevalence of thrombophilias varies significantly among different ethnic groups. These differences may contribute to the apparent contradictions seen in studies that examine the relationship between thrombophilias and adverse pregnancy outcomes.

Acknowledgments We gratefully acknowledge the work of the clinical research midwives, pathology collection staff, and laboratory specimen handlers at both the Mercy Hospital for Women and the Royal Women's Hospital for their invaluable assistance in the recruitment of subjects and sample collection. This project was supported by grants from the Kings Jubilee Fund (City of Melbourne), the Royal Women's Hospital, and the University of Melbourne (Australian Postgraduate Award and Felix Meyer Scholarship).

Received 19 December 2005; revision received 8 June 2006.

Literature Cited

Alfirevic, Z., D. Roberts, and V. Martlew. 2002. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 101(1):6–14.

Angchaisuksiri, P., S. Pingsuthiwong, K. Aryuchai et al. 2000. Prevalence of the G16191A mutation in the factor V gene (factor V Leiden) and the G20210A prothrombin gene mutation in the Thai population. *Am. J. Hematol.* 65(2):119–122.

Awidi, A., M. Shannak, A. Bseiso et al. 1999. High prevalence of factor V Leiden in healthy Jordanian Arabs. *Thromb. Haemost.* 81:582–584.

Bates, S., I. Greer, J. Hirsh et al. 2004. Use of antithrombotic agents during pregnancy: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 126(3):627S–644S.

Chan, D. K., G. Hu, H. Tao et al. 2000. A comparison of polymorphism in the 3′-untranslated region of the prothrombin gene between Chinese and Caucasians in Australia. *Br. J. Haematol.* 111:1253–1255.

Conroy, J. M., G. Trivedi, T. Sovd et al. 2000. The allele frequency of mutations in four genes that confer enhanced susceptibility to venous thromboembolism in an unselected group of New York state newborns. *Thromb. Res.* 99:317–324.

Dahlback, B. 1997. Resistance to activated protein C due to factor V R506Q mutation as a cause of venous thrombosis. *Rev. Invest. Clin.* 49(suppl. 1):3–5.

Dittman, W. A., and P. W. Majeurs. 1990. Structure and function of thrombomodulin: A natural anticoagulant. *Blood* 75(2):329–336.

Esfahani, S. T., E. A. Cogger, and M. A. Caudill. 2003. Heterogeneity in the prevalence of methylenetetrahydrofolate reductase gene polymorphisms in women of different ethnic groups. *J. Am. Diet. Assoc.* 103(2):200–207.

Frosst, P., H. J. Blom, and R. Milos et al. 1995. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* 10:111–113.

Gerhardt, A., R. E. Scharf, M. W. Beckmann et al. 2000. Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. *New Engl. J. Med.* 342(6):374–380.

Gibson, C. S., A. H. MacLennan, Z. Rudzki et al. 2005. The prevalence of inherited thrombophilias in a Caucasian Australian population. *Pathology* 37(2):160–163.

Gudnason, V., D. Stansbie, J. Scott et al. 1998. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (*MTHFR*): Its frequency and impact on plasma homocysteine concentration in different European populations. *Atherosclerosis* 136:347–354.

Herrmann, F. H., M. Koesling, W. Schroder et al. 1997. Prevalence of factor V Leiden mutation in various populations. *Genet. Epidemiol.* 14:403–411.

Infante-Rivard, C., G.-E. Rivard, W. V. Yotov et al. 2002. Absence of association of thrombophilia polymorphisms with intrauterine growth restriction. *New Engl. J. Med.* 347(1):19–25.

Irani-Hakime, N., H. Tamim, R. Kreidy et al. 2000. The prevalence of factor V R506Q mutation-Leiden among apparently healthy Lebanese. *Am. J. Hematol.* 65:45–49.

Isotalo, P. A., G. A. Wells, and J. G. Donnelly. 2000. Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: An examination of C677T and A1298C mutations. *Am. J. Hum. Genet.* 67:986–990.

Lee, D. H., P. A. Henderson, and M. A. Blajchman. 1996. Prevalence of factor V Leiden in a Canadian blood donor population. *Can. Med. Assoc. J.* 155(3):296–298.

Livingston, J. C., J. R. Barton, V. Park et al. 2001. Maternal and fetal inherited thrombophilias are not related to the development of severe preeclampsia. *Am. J. Obstet. Gynecol.* 185(1):153–157.

Livingstone, W. J., C. Keenan, B. White et al. 2000. Prevalence of the factor V R506Q mutation in two Irish control populations: Use of a novel nested polymerase chain reaction approach. *Br. J. Haematol.* 111:559–561.

Poort, S. R., F. R. Rosendaal, P. H. Reitsma et al. 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(10):3698–3703.

Rees, D. C., M. Cox, and J. B. Clegg. 1995. World distribution of factor V Leiden. *Lancet* 346:1133–1134.

Rey, E., S. R. Kahn, M. David et al. 2003. Thrombophilic disorders and fetal loss: A meta-analysis. *Lancet* 361(9361):901–908.

Ridker, P. M., J. P. Miletich, C. H. Hennekens et al. 1997. Ethnic distribution of factor V Leiden in 4,047 men and women: Implications for venous thromboembolism screening. *J. Am. Med. Assoc.* 277(16):1305–1307.

Rosendaal, F. R., C. J. M. Doggen, A. Zivelin et al. 1998. Geographic distribution of the 20210 G to A prothrombin variant. *Thromb. Haemost.* 79:706–708.

Said, J., and G. Dekker. 2003. Pre-eclampsia and thrombophilia. *Best Pract. Res. Clin. Obstet. Gynaecol.* 17(3):441–458.

van der Put, N. M. J., F. Gabreels, E. M. B. Stevens et al. 1998. A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural-tube defects? *Am. J. Hum. Genet.* 62:1044–1051.

Zivelin, A., J. H. Griffin, X. Xu et al. 1997. A single genetic origin for a common Caucasian risk factor for venous thrombosis. *Blood* 89(2):397–402.