

The endothelial protein C receptor (*PROCR*) Ser219Gly variant and risk of common thrombotic disorders: a HuGE review and meta-analysis of evidence from observational studies

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The endothelial protein C receptor (EPCR) limits thrombus formation by enhancing activation of the protein C anticoagulant pathway, and therefore may play a role in the etiology of thrombotic disorders. The rs867186 single-nucleotide polymorphism in the *PROCR* gene (g.6936A > G, c.4600A > G), resulting in a serine-to-glycine substitution at codon 219, has been associated with reduced activation of the protein C pathway, although its association with thrombosis risk remains unclear. The

present study is a highly comprehensive systematic review and meta-analysis, including unpublished genome-wide association study results, conducted to evaluate the evidence for an association between rs867186 and 2 common thrombotic outcomes, venous thromboembolism (VTE) and myocardial infarction (MI), which are hypothesized to share some etiologic pathways. MEDLINE, EMBASE, and HuGE Navigator were searched through July 2011 to identify relevant

epidemiologic studies, and data were summarized using random-effects meta-analysis. Twelve candidate genes and 13 genome-wide association studies were analyzed (11 VTE and 14 MI, including 37 415 cases and 84 406 noncases). Under the additive genetic model, the odds of VTE increased by a factor of 1.22 (95% confidence interval, 1.11-1.33, $P < .001$) for every additional copy of the G allele. No evidence for association with MI was observed. (*Blood*. 2012;119(10):2392-2400)

Introduction

Protein C (PC) is a major component of the coagulation/fibrinolysis cascade. Circulating in plasma as an inactive zymogen, PC is activated at the endothelial surface by the membrane-bound thrombin-thrombomodulin complex.¹ When activated PC (APC) is bound to its cofactor, protein S, it inactivates the procoagulant factors FVa and FVIIIa, limiting the coagulation cascade and fibrin formation.^{1,2} PC activation is enhanced approximately 20-fold when PC binds to the endothelial PC receptor (EPCR),³ a type I transmembrane protein. EPCR is primarily localized on the endothelial cells of large blood vessels (ie, the arteries and veins) and is very sparse or absent in the microvascular endothelium of most tissues.⁴ EPCR-bound APC triggers protease-activated receptor-1 (PAR-1) cleavage, resulting in anti-inflammatory and cytoprotective (eg, antiapoptotic) effects.^{2,5} In addition to its APC-mediated effects, EPCR also works to limit thrombus formation by binding procoagulant FVII/FVIIa, facilitating the clearance of FVIIa and limiting downstream activation of the tissue factor (extrinsic) coagulation pathway.^{6,7} These findings strongly favor an important role for EPCR in thrombosis and inflammation.¹

A soluble form of EPCR (sEPCR) also circulates in the plasma. sEPCR binds PC/APC with the same affinity as membrane-bound EPCR, but does not enhance PC activation by the thrombin-thrombomodulin complex.⁸ Furthermore, sEPCR-bound APC is

incapable of inactivating FVa⁸ and may also impede PAR-1 cleavage.² By limiting APC generation and function, elevated levels of sEPCR may exert procoagulant and proinflammatory effects; in 2 case-control studies,^{9,10} elevated levels of sEPCR were associated with increased risk of VTE. Likewise, a small family study found a higher occurrence of VTE in those with above-normal values of sEPCR compared with those with normal levels.¹¹ The *PROCR* gene is located on chromosome 20q11.2, spans 6 kilobases, and possesses 4 exons.¹² The mature protein comprises 221 amino acids, including an extracellular domain, a 25-amino acid transmembrane domain, and a 3-amino acid intracytoplasmic sequence. Animal experiments have demonstrated the importance of *PROCR* in normal embryonic development; in *PROCR* knock-out mice, fibrin deposition in trophoblast giant cells results in thrombosis at the maternal-embryonic interface.¹³ Death occurs by embryonic day 10.5.

Gene variants and frequency

Mutations in the *PROCR* gene that influence protein expression, function, and/or the concentration of sEPCR may be functionally relevant. Rare point mutations in the gene¹⁴ and its promoter region¹⁵ have been described, but effects on thrombosis and gene expression remain unknown.¹⁶

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The rs867186 diallelic single nucleotide polymorphism in the *PROCR* gene (g.6936A>G, c.4600A>G), resulting in a serine-to-glycine substitution at codon 219 in the membrane-spanning domain of EPCR, explains between 56% and 87% of the variations in sEPCR levels.^{10,17-19} The G allele tags the A3 haplotype (4 *PROCR* haplotypes have been identified in whites) and is associated with increased shedding of EPCR from the endothelial membrane, both by rendering the receptor more sensitive to cleavage²⁰ and by leading to a truncated mRNA through alternative splicing.²¹ The overall frequency of the G allele is 0.074 among individuals included to date in the 1000 Genomes Project²²; however, there are large variations across the population (eg, 0.53 among Papuan New Guineans and 0.0 among South-American Amerindians from the Human Genome Diversity Cell Line Panel).²³ In a genome-wide association study (GWAS) of more than 23 000 cohort participants of European ancestry, the G-allele frequency was 0.101.²⁴

Disease

Venous thromboembolism (VTE) results from an obstruction of blood in the venous system²⁵ by a RBC-rich thrombus composed of platelets and fibrin at sites with low blood flow and shear rate and where the vein wall is normal.²⁶ In contrast, arterial thrombosis (ischemic stroke and coronary artery disease) results from platelet-rich thrombi induced by the rupture of an atherosclerotic plaque at arterial sites where shear rates are high. Although arterial and venous thromboses have traditionally been viewed as distinct, recent studies suggest that the 2 conditions share common etiologic pathways. Evidence in support of this is 3-fold (for review, see Prandoni²⁷ or Lowe²⁶): (1) several observational studies have demonstrated increased risk of subsequent arterial vascular disease among patients with VTE; (2) drugs affecting coagulation and hemostasis have some effect in preventing and treating various atherosclerotic disorders; and (3) VTE and myocardial infarction (MI) share common risk factors (albeit of different magnitudes), including genetic determinants such as the Factor V Leiden (F5L) and prothrombin G20210A gene mutations^{28,29} and variants in the *ABO* gene,^{30,31} as well as age, obesity, hypertension, diabetes mellitus, smoking, and hypercholesterolemia.³² All of these risk factors increase susceptibility to thrombus formation by modifying the coagulation and fibrinolytic systems.³³

The *PROCR* rs867186 variant has emerged as a candidate risk factor for both arterial and venous thrombotic disease because of the involvement of EPCR in APC- and FVII/FVIIa-mediated clotting and inflammation. However, evidence for an association between the *PROCR* rs867186 variant and arterial and venous thrombosis has been conflicting. We therefore undertook a systematic review and meta-analysis of observational studies to evaluate the evidence for an association between the *PROCR* rs867186 variant and 2 common thrombotic outcomes, VTE and MI, which are hypothesized to share etiologic pathways. Although ischemic stroke is also a common thrombotic outcome, we chose not to include it in our review because the outcome was highly variable (ie, ischemic and hemorrhagic stroke cases were often indistinguishable) and because the number of studies of stroke was low.

Methods

Search strategy

MEDLINE, EMBASE, and the HuGE Navigator Genopedia database were searched through July 2011 using a combination of keywords, including

“thromboembolism,” “thrombosis,” “pulmonary embolism,” “myocardial infarction,” “EPCR,” and “PROCR” (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Titles and abstracts of identified articles were screened by 2 reviewers, and those that discussed any *PROCR* variant in the context of VTE or MI proceeded to a full-text screening. The full text of articles was screened by 2 reviewers and case-control, cohort, or cross-sectional studies examining an association between the *PROCR* rs867186 polymorphism and VTE or MI were selected for inclusion, with disagreements resolved by consensus. Reference lists of included studies and review articles were searched for additional articles. Genetic linkage studies, review articles, animal studies, and conference proceedings were excluded.

GWAS of VTE or MI in which the *PROCR* rs867186 variant, or variants in close linkage disequilibrium with rs867186, might potentially have been included on the genotyping platform were located using keyword searches of the HuGE Navigator GWAS Integrator database. Unpublished GWAS were identified by keyword searches of the National Center for Biotechnology Information database of genotypes and phenotypes (dbGaP). Authors of relevant GWAS were contacted to request counts of events and nonevents by *PROCR* rs867186 genotype (ultimately, all identified GWAS had genotyped this single-nucleotide polymorphism).

Information on study design, location, demographics, ascertainment of subjects, case definition, and genotype and allele frequencies was extracted from included studies by 2 independent reviewers using a standardized data abstraction form. When this information was not available from the article, it was sought from other publications reporting on the same study population, if available. Crude odds ratios (ORs) and their 95% confidence intervals (95% CIs) were calculated from genotype frequencies presented in the published article; when separate genotype counts were not presented, they were requested from the study authors.

Meta-analysis

Inverse variance-weighted random effects meta-analysis was used to estimate the summary effect and 95% CI for VTE, MI, and VTE and MI combined, using Stata Version 11 software. The G allele was considered the at-risk allele, and the per-allele effect estimate was calculated as the OR per unit score (0, 1, or 2 copies of the G allele) using logistic regression. The per-allele OR is the risk of disease per one-allele increase, and the *P* value of the OR tests the hypothesis of zero slope for a line that best fits the 3 genotypic risk estimates.³⁴ The per-allele model is powerful for detecting additive genetic effects³⁴ and the additive model is in accordance with the observed variation in sEPCR values according to the number of copies of the G allele.^{18,19,35} In secondary analyses, 4 additional genotype contrasts were tested for each outcome: AG versus AA, GG versus AA, GG + AG versus AA (dominant model), and GG versus AG + AA (recessive model). The intensity and significance of between-study heterogeneity were assessed with, respectively, the *I*² statistic with its 95% uncertainty interval, and the Cochran *Q* statistic. *I*² values of 25%, 50%, and 75% indicate low, medium, and high between-study heterogeneity, respectively, and *P* > .05 for the Cochran *Q* statistic suggests no statistically significant heterogeneity.³⁶ Publication bias was assessed graphically using funnel plots and the Egger test quantified the asymmetry of the plot. The latter tests the null hypothesis that small studies give the same results as large studies³⁷; *P* < .05 was deemed statistically significant.

Four subgroup analyses were subsequently carried out to explore possible explanations for heterogeneity. In the first, GWAS were excluded from the meta-analysis; in the second, candidate gene studies were excluded from the meta-analysis; in the third, analyses were restricted to studies of white populations; and in the fourth, studies with deviation from Hardy-Weinberg equilibrium (HWE), a potential indicator of poor genotyping quality or ascertainment issues, were removed. HWE was investigated in controls of case-control studies and in the entire samples of cohort studies using the standard χ^2 goodness of fit test as well as the relative excess heterozygosity (REH).³⁸ The χ^2 goodness-of-fit statistic tests the null hypothesis that the data are consistent with HWE. In contrast, the REH approach quantifies Hardy-Weinberg disequilibrium by measuring the ratio of the actual proportion of heterozygotes to the proportion of heterozygotes expected in a population, which conforms to HWE.³⁸ A significant

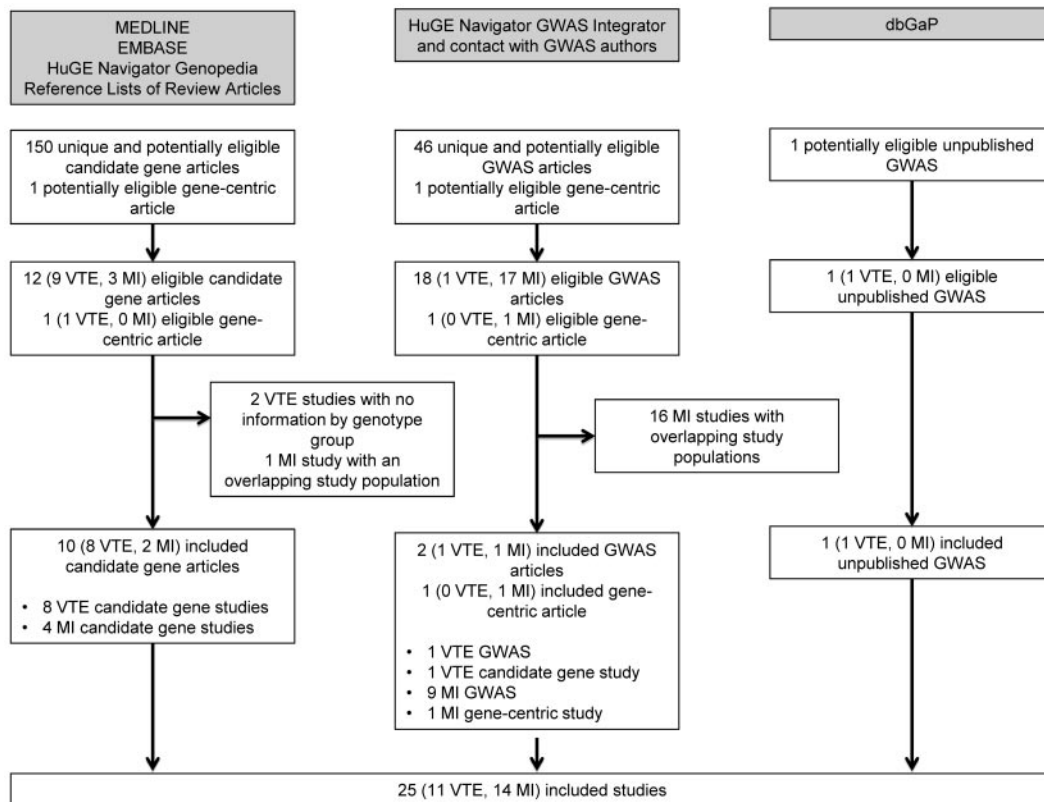


Figure 1. Articles and studies identified from a systematic search of the literature on the *PROC1* rs867186 polymorphism and venous and arterial thrombosis.

deviation of the ratio from unity, as measured by the 2-sided 95% CI, is evidence of Hardy-Weinberg disequilibrium. Among studies of MI, a fifth subgroup analysis was carried out in which analysis was restricted to studies that had used a history of MI as the case definition.

The Venice criteria for assessing the strength of cumulative evidence in genetic association studies were used.³⁹ Briefly, this semiquantitative index classifies the credibility of cumulative epidemiologic evidence into 3 categories, “weak,” “moderate,” and “strong,” taking into consideration criteria such as amount of evidence (eg, sample size, power, and false-discovery rate), replication (eg, attention to phenotype definition, models, and I^2), and protection from bias (eg, population stratification and measurement errors). This review was prepared following the guidance of the *HuGE Review Handbook* Version 1.0.⁴⁰

Results

The search of MEDLINE, EMBASE, and HuGE Navigator Genopedia returned 150 unique candidate gene articles, of which 12 (9 VTE and 3 MI) were eligible for inclusion (Figure 1). The study populations overlapped in 3 studies of VTE,^{9,17,41} so de-duplicated counts were obtained from one of the authors and included. The population in one study of MI¹⁹ overlapped with that in the gene-centric meta-analysis⁴² included in the systematic review (see next paragraph), so this article was excluded from further analysis. A second candidate gene article,⁴³ as well as a gene-centric study of VTE identified from the reference list of a review paper,⁴⁴ was excluded because genotype counts were not available separately for each of the 3 genotype groups.

The search of HuGE Navigator GWAS Integrator returned 46 GWAS, of which 18 (1 VTE and 17 MI) were eligible for inclusion (Figure 1). Contact with GWAS authors resulted in the identification of an additional gene-centric (> 2000 genes) study

not yet published when our search was executed.⁴² This study, as well as the most recent GWAS of MI,³¹ were meta-analyses that included 21 studies. These 2 studies encompassed almost all of the previously published GWAS results; therefore, no other GWAS of MI beyond these 2 meta-analyses were included in this systematic review. Study-specific genotype counts were obtained from the GWAS meta-analysis³¹ for use in the present meta-analysis. In the gene-centric meta-analysis,⁴² because the per-allele OR was presented but genotype counts were not, the per-allele OR was used in the present meta-analysis. One additional unpublished GWAS of VTE was identified from dbGaP (J.A.H. and M.d.A., unpublished data, March 21, 2011; referred to hereafter as “Heit 2011”) for a total of 2 included GWAS of VTE. The published GWAS of VTE³⁰ also included a candidate gene study that had not been identified in previous searches.

Overall, the systematic review included 25 studies (11 VTE and 14 MI) for a total of 37 415 cases (4821 with VTE and 32 594 with MI) and 84 406 noncases (6070 VTE noncases and 78 336 MI noncases).

Characteristics of studies included in the meta-analysis are presented in supplemental Table 2. All 11 VTE studies used a case-control design, of which 2^{9,41} were family-based (controls were first-degree relatives of cases), one⁴⁵ was nested within a cohort study, and 2 (Tregouet et al³⁰ and Heit 2011), were GWAS designs. Six studies^{9,17,30,35,41} included white subjects only, 3 studies (Yamagishi et al,⁴⁵ Pecheniuk et al,⁴⁶ and Heit 2011) included white and nonwhite subjects, one study⁴⁷ included Chinese subjects, and one study¹⁰ did not report the race/ethnicity of subjects. Cases and controls were matched by age and gender in 6 studies (Uitte de Willige et al,¹⁰ Saposnik et al,³⁵ Yamagishi et al,⁴⁵ Pecheniuk et al,⁴⁶ Chen et al,⁴⁷ and Heit 2011). In all but 2 non-matched studies, age

Table 1. Distribution of PROCR rs867186 genotypes among cases and non-cases in studies of VTE and MI

Included study*	AA genotype				AG genotype				GG genotype				MAF†	Deviation from Hardy-Weinberg equilibrium	
	Cases		Non-cases		Cases		Non-cases		Cases		Non-cases			χ ² P value	REH (95% CI)‡
	n	%	n	%	n	%	n	%	n	%	n	%			
VTE															
Medina 2004 ¹⁷	291	82	327	82	62	17	74	18	2	1	0	0	0.09	.04	
Saposnik 2004 ³⁵	249	74	278	82	85	25	58	17	4	1	2	1	0.09	.58	1.23 (0.59-2.58)
Uitte de Willige 2004 ¹⁰	345	73	361	77	116	25	100	21	10	2	10	2	0.13	.33	0.83 (0.58-1.21)
Medina 2005 ⁴¹	77	81	145	80	17	18	35	19	1	1	1	1	0.10	.47	1.45 (0.52-4.10)
Navarro 2008 ⁹	58	69	128	86	24	29	21	14	2	2	0	0	0.07	.35	
Pecheniuk 2008 ⁴⁶	82	72	87	76	27	24	24	21	5	4	3	3	0.13	.40	0.74 (0.37-1.50)
Trégouët 2009–GWAS ³⁰	309	75	1003	82	92	22	216	18	9	2	8	1	0.09	.32	1.21 (0.83-1.75)
Trégouët 2009–MARTHA ³⁰	885	79	654	82	222	20	141	18	16	1	5	1	0.09	.38	1.23 (0.77-1.97)
Yamagishi 2009 ⁴⁵	417	84	844	83	72	15	158	16	7	1	14	1	0.09	.04	0.73 (0.53-0.99)
Chen 2011 ⁴⁷	49	75	63	89	15	23	7	10	1	2	1	1	0.06	.15	0.44 (0.13-1.52)
Heit 2011	978	77	1029	79	264	21	257	20	28	2	16	1	0.11	.99	1.00 (0.76-1.32)
MI															
Ireland 2005–EDSC-EW ¹⁸	33	65	231	85	17	33	37	14	1	2	4	1	0.08	.09	0.61 (0.34-1.10)
Ireland 2005–EDSC-IA ¹⁸	63	55	222	64	48	42	108	31	3	3	19	5	0.21	.23	0.83 (0.62-1.12)
Ireland 2005–HIFMECH ¹⁸	440	84	455	81	83	16	101	18	3	1	7	1	0.10	.60	0.89 (0.59-1.36)
Ireland 2005–NPHSII ¹⁸	166	85	1883	82	26	13	387	17	4	2	14	1	0.09	.57	1.08 (0.84-1.38)
Medina 2008 ⁴⁸	606	88	554	79	80	12	142	20	3	0	1	0	0.10	.01	3.02 (1.12-8.16)
Schunkert 2011–ADVANCE ³¹	221	79	262	84	55	20	49	16	2	1	1	0	0.08	.41	1.51 (0.55-4.20)
Schunkert 2011 CADomics ³¹	1642	79	2324	79	409	20	589	20	25	1	37	1	0.11	.96	1.00 (0.84-1.20)
Schunkert 2011 deCODE CAD ³¹	5509	83	22 711	82	1078	16	4640	17	51	1	244	1	0.09	.68	0.99 (0.92-1.06)
Schunkert 2011 GerMIFS I ³¹	670	77	1225	77	184	21	344	22	11	1	23	1	0.12	.84	1.02 (0.81-1.29)
Schunkert 2011 GerMIFS II ³¹	981	80	1007	78	227	19	265	21	14	1	15	1	0.11	.60	1.08 (0.81-1.43)
Schunkert 2011 GerMIFS III ³¹	922	80	1359	78	222	19	360	21	13	1	29	2	0.12	.36	0.91 (0.73-1.12)
Schunkert 2011 MedStar ³¹	369	83	721	82	76	17	148	17	2	0	6	1	0.09	.59	1.13 (0.73-1.73)
Schunkert 2011 MIGen ³¹	1035	81	1151	82	224	18	240	17	12	1	14	1	0.10	.71	0.95 (0.71-1.27)
Schunkert 2011 OHGS1 ³¹	1207	82	1141	81	257	18	263	19	4	0	11	1	0.10	.33	1.17 (0.85-1.62)
IBC 50K CAD Consortium (European) ^{42§}													0.10		
IBC 50K CAD Consortium (South Asian) ^{42§}													0.19		

*Studies are identified by the lead author's last name, year of publication, study name (if a publication included more than 1 study), and reference number.

†The minor allele (G) frequency (MAF) was calculated in controls or in the underlying cohort.

‡The REH cannot be calculated in studies with 0 cases or controls with the GG genotype.

§Genotype frequencies were not reported in the published article; however, the study authors state that the single-nucleotide polymorphism did not violate HWE in the controls ($P > .0001$).

and gender distributions were similar in cases and controls.³⁰ Two studies were of first-event VTE patients,^{10,30} all cases of VTE were objectively diagnosed, and the case definition included deep vein thrombosis and pulmonary embolism, except in 2 studies^{10,47} that were limited to deep vein thrombosis only. Cases were recruited from thrombosis clinics in 8 studies, from a venous thrombosis registry in one study,⁴⁶ and from a community-based cohort in another study.⁴⁵ Controls were selected from hospital personnel,¹⁷ general medical examination clinics (Trégouët et al,³⁰ Saposnik et al,³⁵ and Heit 2011), partners and acquaintances of cases,¹⁰ first-degree relatives of cases,^{9,41} the community,^{30,46} the underlying cohort,⁴⁵ or from a clinical trial of antioxidant supplementation.³⁰ In one study,⁴⁷ the source of cases and controls was not stated.

The 14 MI studies included 1 cohort study, 3 case-control studies, 9 GWAS case-control studies, and 1 case-control gene-centric meta-analysis (supplemental Table 2). Twelve studies included white subjects only; the remaining 2 studies presented

results for white subjects and South-Asian subjects separately.^{18,42} This stratification by race/ethnicity was maintained in the present meta-analysis for a total of 16 MI studies analyzed. Cases and controls were matched by age and gender in 3 studies,^{18,31,48} whereas the age and/or gender distribution of cases and controls was either not reported or differed considerably in all but 1⁴² of the other studies. One study was of first-event MI patients¹⁸ and the case definition was a history of MI in 6 studies, whereas a composite case definition including coronary intervention procedures, angina, > 50% stenosis of coronary vessels, and MI was used in the other 11 studies. Cases and noncases were population, hospital, or clinic based.

Genotype and minor allele frequency distributions were similar between studies (Table 1), with the exception of the studies of South Asians. Whereas the frequency of the G allele among controls or in the underlying cohort was 13% or less in white populations, the frequency was 19%-21% among South Asian controls. There was evidence of deviation from HWE in 3 studies

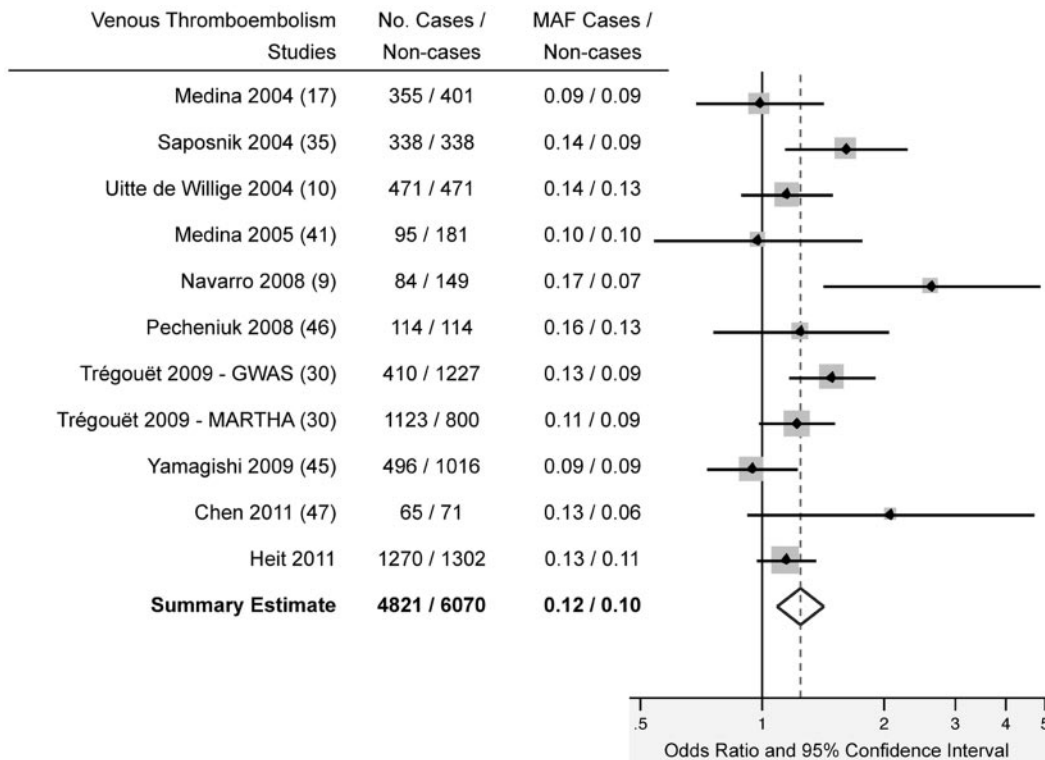


Figure 2. Results from a random-effects meta-analysis of the association between the *PROCR* rs867186 polymorphism and VTE (per-allele model). Individual studies are identified by the lead author's last name, year of publication, study name (if a publication included more than 1 study), and reference number. The sample sizes are represented by the size of the squares. Bars indicate 95% CI.

(2 of VTE^{17,45} and one of MI⁴⁸), with both methods of HWE assessment yielding similar conclusions.

VTE

The meta-analysis supported an association between the *PROCR* rs867186 variant and VTE (Figure 2 and Table 2), with the odds of VTE increasing by a factor of 1.22 (95% CI, 1.11-1.33) for every additional copy of the G allele ($P < .001$). A statistically significant association was also observed for the dominant model, the recessive model, and for the GG-versus-AA contrast, whereas the AG-versus-AA contrast approached statistical significance. Between-study heterogeneity was low under the per-allele model (20%, 95% uncertainty interval: 0%-52%), but was moderate for the AG-versus-AA contrast and for the dominant model. Although between-study heterogeneity was low under the recessive model and for the GG-versus-AA

contrast, this was likely the result of poor precision due to the small number of GG subjects and should not be interpreted as a lack of true between-study heterogeneity.³⁶ Under the per-allele model, the funnel plot (supplemental Figure 1) and the Egger test ($P = .23$) revealed no evidence of publication bias. Subgroup analyses yielded results similar to those obtained from analysis of all subjects (Table 3).

Of the individual studies included in the meta-analysis, the largest association between the *PROCR* rs867186 variant and VTE was observed in a study⁹ of prothrombin G20210A mutation carriers (per-allele OR = 2.63; 95% CI, 1.41-4.87). Although no other included study was conducted exclusively among these mutation carriers, 7 other studies reported the prevalence of G20210A mutation carriers; the prevalence was less than 25% and no obvious gradient in the estimated ORs according to G20210A mutation prevalence was observed (supplemental Figure 2 and

Table 2. Associations between *PROCR* rs867186 genotypes and VTE or MI in a random-effects meta-analysis

Genotype contrast	Included studies, n	Total cases, n	Total non-cases, n	OR	95% CI	I ²	95% Uncertainty interval	Cochran Q P value
VTE								
Per-allele model	11	4821	6070	1.22	1.11-1.33	20	0-52	.197
AG versus AA	11	4736	6010	1.21	1.05-1.40	43	0-72	.063
GG versus AA	11	3825	4979	1.81	1.29-2.56	0	0-46	.694
AG + GG versus AA (dominant)	11	4821	6070	1.25	1.08-1.44	47	0-74	.041
GG versus AG + AA (recessive)	11	4821	6070	1.76	1.24-2.48	0	0-42	.739
MI								
Per-allele model	16	32 594	78 336	0.94	0.88-1.00	65	40-79	< .001
AG versus AA	14	16 850	42 919	0.96	0.86-1.06	67	43-81	< .001
GG versus AA	14	14 012	35 671	0.87	0.72-1.06	0	0-53	.502
AG + GG versus AA (dominant)	14	16 998	43 344	0.95	0.86-1.05	65	37-80	< .001
GG versus AG + AA (recessive)	14	16 998	43 344	0.88	0.72-1.06	0	0-55	.462

Table 3. Associations within subgroups between *PROCR* rs867186 genotypes and VTE or MI under the per-allele model in a random-effects meta-analysis

Subgroup	Included studies, n	Total cases, n	Total non-cases, n	OR	95% CI	I ²	95% Uncertainty interval	Cochran Q P value
VTE								
Candidate gene studies	9	2428	3968	1.19	1.07-1.34	15	0-51	.271
GWAS	2	1680	2529	1.28	1.02-1.61	29	0-74	.240
Studies of white subjects only	6	2405	3096	1.35	1.14-1.59	12	0-53	.321
Studies in HWE	9	3970	4653	1.28	1.16-1.41	10	0-46	.333
MI								
Candidate gene studies	5	1576	4165	1.03	0.68-1.56	85	66-93	< .001
GWAS	11	31 018	74 171	0.93	0.89-0.98	36	0-68	.113
Studies of white subjects only	14	28 086	73 728	0.93	0.86-1.00	66	40-81	< .001
Studies in HWE	15	31 905	77 639	0.95	0.90-1.01	54	16-74	.007
Studies of subjects with a history of MI only	6	5730	7292	0.87	0.75-1.00	64	14-85	.016

supplemental Table 3). One included study was conducted exclusively among F5L mutation carriers.⁴¹ Results from this study were null (per-allele OR = 0.98; 95% CI, 0.54-1.77). A large positive association (per-allele OR = 1.49; 95% CI, 1.17-1.90) was observed in the study that included only idiopathic VTE subjects (ie, VTE in the absence of acquired risk factors: surgery, hospitalization, pregnancy, puerperium, oral contraception, cancer, and autoimmune disease; and in the absence of strong known genetic risk factors: antithrombin, protein S, or PC deficiencies, and homozygosity for F5L or prothrombin G20210A).³⁰ In the 7 other studies in which the etiology of VTE was reported, the proportion of idiopathic VTE subjects ranged from 25%-65% and there was no obvious trend in the estimated ORs according to the frequency of idiopathic subjects.

MI

The meta-analysis did not support an association between the *PROCR* rs867186 variant and MI (Figure 3 and Table 2). None of the investigated genotype contrasts reached statistical significance. Heterogeneity between MI studies under all genetic models was moderate to high (again, the apparent low heterogeneity under the recessive model and for the GG-versus-AA contrast is likely the result of wide CIs in analyses of GG subjects). No publication bias under the per-allele model was observed (supplemental Figure 1), with an Egger test *P* = .25. When the analysis was restricted to GWAS, under the per-allele model, the I² was reduced to 36% (95% uncertainty interval, 0%-68%) and the OR for MI was 0.93 (95% CI, 0.89-0.98) for every additional copy of the G allele (*P* = .005; Table 3). In all other subgroup analyses, heterogeneity remained

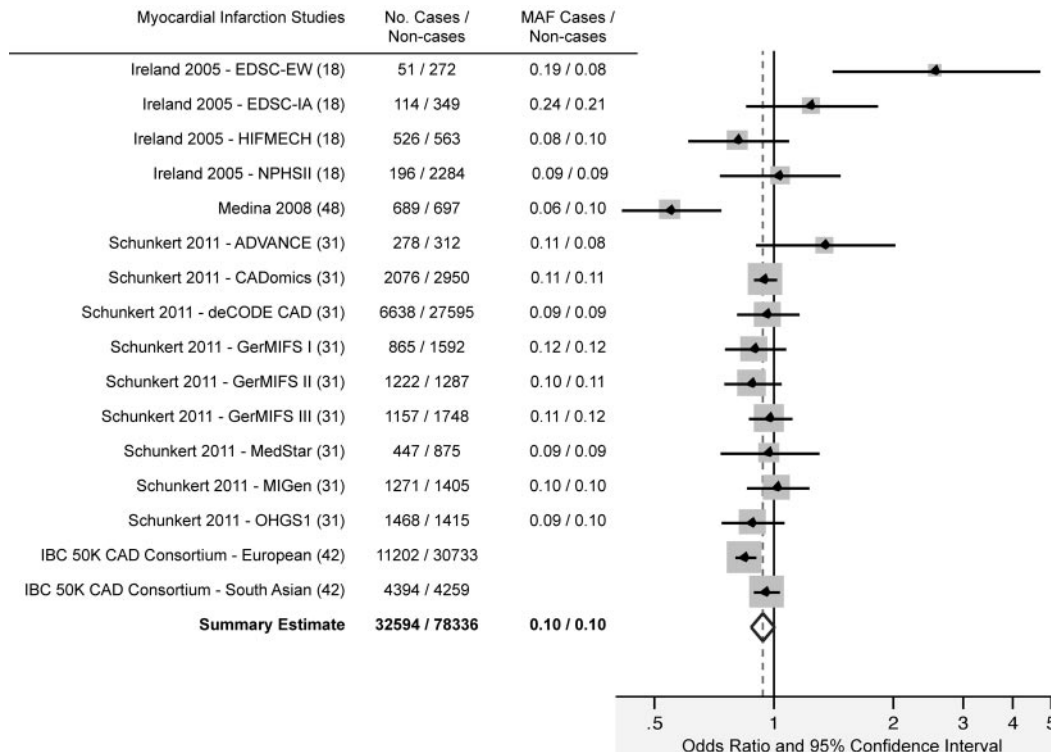


Figure 3. Results from a random-effects meta-analysis of the association between the *PROCR* rs867186 polymorphism and MI (per-allele model). Individual studies are identified by the lead author's last name, year of publication, study name (if a publication included more than 1 study), and reference number. The sample sizes are represented by the size of the squares. Bars indicate 95% CI. Allele frequencies were not reported in the IBC 50K CAD Consortium article.

moderate to high and the effect estimate was similar to that obtained from analysis of all subjects.

Of the 16 included studies of MI, a statistically significant association between increasing copies of the *PROCR* rs867186 G allele and MI was found in 3; in 1 of these, the association was positive, and in the other 2, there was an inverse association. The statistically significant positive association was observed in the study¹⁸ of white diabetics (per-allele OR = 2.57; 95% CI, 1.41-4.69). Among South-Asian diabetics, the association was also positive, although not statistically significant (per-allele OR = 1.25; 95% CI, 0.85-1.83). The largest inverse association was found in a Spanish study⁴⁸ of subjects with a history of MI (per-allele OR = 0.55; 95% CI, 0.41-0.74). However, HWE was violated in this study and the results may not be comparable to other included studies. The third statistically significant result was observed in the gene-centric meta-analysis of European subjects⁴² comprising 10 studies (per-allele OR = 0.85; 95% CI, 0.80-0.90).

Although initially intended, results of VTE and MI studies were not pooled together in a combined analysis because of differences in the direction of the association with the *PROCR* rs867186 G allele in VTE and MI studies.

Discussion

This meta-analysis of 4821 VTE cases and 6070 controls found a significant association between the *PROCR* rs867186 variant and VTE. Under an additive genetic model, the odds of VTE increased by 22% for every additional copy of the G allele. In contrast, the meta-analysis found no association between the *PROCR* rs867186 variant and MI. With nearly 17 000 MI cases and more than 43 000 noncases, and based on our current knowledge of trait and variant characteristics (ie, disease prevalence of approximately 4%,⁴⁹ population G allele frequency of approximately 10%,²⁴ and an additive disease model), the meta-analysis had > 99% power to detect a per-allele effect as small as 1.1 at $P = .05$ (supplemental Table 4),⁵⁰ if such an association existed. However, substantial between-study heterogeneity and other population-specific characteristics may have reduced this theoretical power. Given the present results, the hypothesis of some common pathways underlying venous and arterial thrombosis may not apply to the *PROCR* gene and the variant may be a genetic risk factor for VTE only.

VTE

The association with VTE is in accordance with the proposed functional implications of the *PROCR* rs867186 variant, namely, the G allele, causes EPCR shedding from the endothelial membrane, reduced PC activation, and higher FVII/FVIIa levels, eventually leading to thrombosis. Of the 8 studies included in the meta-analysis that examined levels of sEPCR relative to the *PROCR* rs867186 variant, all observed a significant increase associated with the G allele.^{9,10,17,18,35,45,47,48} Four studies reported levels of PC or APC by genotype. Of these, two^{19,45} observed a statistically significant increase in PC associated with the G allele, one⁹ observed a statistically significant decrease in APC among G allele carriers, and one¹⁷ found no association between genotype and APC levels. In a GWAS of plasma levels of PC including more than 8000 participants, the strongest association was observed at the *PROCR* locus, and the rs867186 polymorphism explained an estimated 10.4% of the variance in PC.⁵¹ In 2 studies included in the systematic review that measured prothrombin (fragment 1 and

2) levels relative to genotype, a significant increase was seen for those with the G allele.^{17,18} The G allele was also associated with elevated levels of FVII and FVIIa in a study of approximately 2000 healthy middle-aged men.⁶

VTE is multicausal, in that multiple genetic and environmental factors contribute to its etiology.⁵² Two genetic risk factors for VTE with relatively high population prevalence are well established: the F5L and the prothrombin G20210A mutations. The largest association between the *PROCR* rs867186 variant and VTE identified in our review was observed in a study of prothrombin G20210A mutation carriers.⁹ However, further investigation of the modifying role of this mutation was hampered by the low prevalence—or incomplete reporting of the prevalence—of prothrombin G20210A mutation carriers in the other included studies. No association between the *PROCR* rs867186 variant and VTE was observed in 2 studies of F5L mutation carriers (only 1 of which was included in the review).^{41,43} In a study (not included in the review) of 2 families with inherited thrombophilia, a stronger association with VTE was observed in those with both the *PROCR* G allele and a dysfunctional PC gene variant relative to those with a dysfunctional PC gene variant alone.¹¹ Although limited by the small number of studies, these findings suggest that the *PROCR* rs867186 polymorphism may act in concert with some known genetic risk factors, but not others, to increase VTE risk.

All but 1 study included in the meta-analysis³⁰ used a pooled definition of idiopathic and non-idiopathic VTE cases; only 1 study restricted the case definition to idiopathic VTE patients. In pooling idiopathic and non-idiopathic VTE cases, heterogeneity in the definition of the outcome may have weakened or even masked the effect of the *PROCR* rs867186 variant on VTE. Providing some evidence for this is the fact that the study restricted to idiopathic VTE patients reported a large positive association with the G allele of *PROCR* rs867186.

MI

Between-study heterogeneity in MI studies was moderate to high, as indicated by both the I^2 and Cochran Q statistics, and is likely attributable to differences in study design, participant selection, and participant characteristics. The meta-analysis pooled results of cohort, case-control, and GWAS designs; in 10 studies, the case definition included MI grouped with other coronary heart diseases, and the severity of MI (fatal versus nonfatal) varied or was unclear across studies. Two studies were restricted to men, some studies included younger subjects (< 60 years of age), whereas the age and gender of subjects was not reported in the study of diabetics. The I^2 was less among GWAS, but even among the 9 GWAS in which information on subject characteristics was available, the frequency of cases with a history of MI ranged from 48%-100%, the age and gender distribution of subjects varied considerably, and 2 studies were restricted to cases with a family history of coronary artery disease.

Although no overall association with MI was found, when analysis was restricted to GWAS under the per-allele model, a statistically significant inverse association with the *PROCR* rs867186 G allele emerged. This finding may be because of reduced heterogeneity in GWAS as a result of harmonization of the case definition, quality control measures, and analysis methods that occurs in consortiums, of which all of the GWAS were part. Our GWAS findings are consistent with a recently published (ie, after our search was updated for the last time) gene-centric case-control study of Italian early-onset MI cases⁵³; however, they are nonetheless difficult to explain. The biologic mechanism underlying an

inverse association between rs867186 and MI is unclear. It may be that the *PROCR* rs867186 G allele is in linkage disequilibrium with the true causal variant.

Strengths and limitations

Strengths of the systematic review include the thorough search for articles, the investigation of both venous and arterial thrombotic disease, the large number of included subjects, and the use of a genetic model that is based on biologic evidence. The limitations of the meta-analysis include the possible influences of selection bias and genotype and phenotype misclassification on the results of individual studies and on the results of the meta-analysis, which are difficult to predict. Bias could also have arisen when calculating the crude ORs from genotype frequencies, because this could not be taken into consideration in the analysis when cases and controls were matched. Nonetheless, unmatched effect estimates were almost identical to those presented in the published article, either because matched and unmatched analyses produced similar results or because the investigators had not performed a matched analysis. When estimates adjusted for potential confounders were presented in the published article, these too approximated the crude estimates calculated from genotype frequencies. A major source of confounding in genetic association studies, population stratification, was assessed in the meta-analysis by restricting analysis to white subjects only. Although confounding was not detected, the “white” designation may comprise individuals of various races/ethnicities, and hidden population stratification cannot be completely ruled out.

Whereas selective reporting must be considered in all meta-analyses, because of ready sample availability and low genotyping cost, it is especially a concern in studies of gene-disease associations, in which authors may test many polymorphisms but only report the most interesting or statistically significant findings. Even the means by which publication bias is measured, funnel plots and statistical tests of funnel plot asymmetry, have limited power.⁴⁰ Although no statistically significant publication bias was detected, it is nonetheless possible that studies with null findings were not published and are missing from the systematic review. In an attempt to overcome this limitation, we included in our search both published and unpublished GWAS data, and authors of all eligible studies were contacted to request genotype counts.

Conclusions

Applying the Venice criteria,³⁹ the strength of cumulative evidence for an association between the *PROCR* rs867186 variant and VTE is “moderate.” The meta-analysis had > 80% power to detect a per-allele relative risk of 1.15 (supplemental Table 4), there existed some between-study heterogeneity, and the effect of potential

biases could not be completely assessed. This corresponds to a score of “B” for amount of evidence, replication, and protection from bias. Using the same criteria, the strength of evidence for an association with MI is “weak.” Despite a considerable sample size indicating large-scale evidence (an “A” rating), there existed substantial between-study inconsistency in MI phenotype definition, study populations, and study findings, and a null overall effect was found (a “C” rating for replication).

In light of the ever-increasing number of gene-disease association studies, especially in the postGWAS era, HuGE reviews are a useful tool to summarize this data. Overall, there is moderate evidence for an association between the *PROCR* rs867186 polymorphism and VTE, whereas there is weak evidence for an association with MI. This suggests that a common mechanism underlying venous and arterial thrombosis is not mediated by the *PROCR* rs867186 polymorphism.

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Authorship

Contribution: F.G., P.E.M., and D.-A.T. conceived the research; J.D., F.G., and C.Y.J. designed the study; J.D. led the implementation, analysis, and interpretation of the study; A.S.A. screened articles for eligibility and extracted the data; M.d.A. and J.A.H. contributed to data acquisition; J.D. drafted the manuscript; and all authors critically reviewed the manuscript for intellectual content.

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References

- Geiger M. Soluble protein C receptor: why? *Blood*. 2008;111(7):3301-3302.
- Gandridge S. Endothelial cell protein C receptor and the risk of venous thrombosis. *Haematologica*. 2008;93(6):812-816.
- Stearns-Kurosawa DJ, Kurosawa S, Mollica JS, Ferrell GL, Esmon CT. The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin complex. *Proc Natl Acad Sci U S A*. 1996;93(19):10212-10216.
- Laszik Z, Mitro A, Taylor FB, Jr Ferrell G, Esmon CT. Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway. *Circulation*. 1997;96(10):3633-3640.
- Mosnier LO, Zlokovic BV, Griffin JH. The cytoprotective protein C pathway. *Blood*. 2007;109(8):3161-3172.
- Ireland HA, Cooper JA, Drenos F, et al. FVII, FVIIa, and downstream markers of extrinsic pathway activation differ by EPCR Ser219Gly variant in healthy men. *Arterioscler Thromb Vasc Biol*. 2009;29(11):1968-1974.
- Ghosh S, Pendurthi UR, Steinoe A, Esmon CT, Mohan Rao LV. Endothelial cell protein C receptor acts as a cellular receptor for factor VIIa on endothelium. *J Biol Chem*. 2007;282(16):11849-11857.
- Liaw PC, Neuenschwander PF, Smirnov MD, Esmon CT. Mechanisms by which soluble endothelial cell protein C receptor modulates protein C and activated protein C function. *J Biol Chem*. 2000;275(8):5447-5452.
- Navarro S, Medina P, Mira Y, et al. Haplotypes of the EPCR gene, prothrombin levels, and the risk of venous thrombosis in carriers of the prothrombin G20210A mutation. *Haematologica*. 2008;93(6):885-891.
- Uitte de Willige S, Van Marion V, Rosendaal FR,

- Vos HL, De Visser MCH, Bertina RM. Haplotypes of the EPCR gene, plasma sEPCR levels and the risk of deep venous thrombosis. *J Thromb Haemost.* 2004;2(8):1305-1310.
11. Simioni P, Morboeuf O, Tognin G, et al. Soluble endothelial protein C receptor (sEPCR) levels and venous thromboembolism in carriers of two dysfunctional protein C variants. *Thromb Res.* 2006;117(5):523-528.
 12. Simmonds RE, Lane DA. Structural and functional implications of the intron/exon organization of the human endothelial cell protein C/activated protein C receptor (EPCR) gene: Comparison with the structure of CD1/major histocompatibility complex alpha1 and alpha2 domains. *Blood.* 1999;94(2):632-641.
 13. Gu JM, Crawley JT, Ferrell G, et al. Disruption of the endothelial cell protein C receptor gene in mice causes placental thrombosis and early embryonic lethality. *J Biol Chem.* 2002;277(45):43335-43343.
 14. Biguzzi E, Merati G, Liaw PCY, et al. A 23bp insertion in the endothelial protein C receptor (EPCR) gene impairs EPCR function. *Thromb Haemost.* 2001;86(4):945-948.
 15. Biguzzi E, Gu J, Merati G, Esmon NL, Esmon CT. Point mutations in the endothelial protein C receptor (EPCR) promoter. *Thromb Haemost.* 2002;87(6):1085-1086.
 16. Medina P, Navarro S, Estelles A, Espana F. Polymorphisms in the endothelial protein C receptor gene and thrombophilia. *Thromb Haemost.* 2007;98(3):564-569.
 17. Medina P, Navarro S, Estelles A, et al. Contribution of polymorphisms in the endothelial protein C receptor gene to soluble endothelial protein C receptor and circulating activated protein C levels, and thrombotic risk. *Thromb Haemost.* 2004;91(5):905-911.
 18. Ireland H, Konstantoulas CJ, Cooper JA, et al. EPCR Ser219Gly: elevated sEPCR, prothrombin F1 +2, risk for coronary heart disease, and increased sEPCR shedding in vitro. *Atherosclerosis.* 2005;183(2):283-292.
 19. Reiner AP, Carty CL, Jenny NS, et al. PROC, PROCr and PROC1 polymorphisms, plasma anticoagulant phenotypes, and risk of cardiovascular disease and mortality in older adults: The Cardiovascular Health Study. *J Thromb Haemost.* 2008;6(10):1625-1632.
 20. Qu D, Wang Y, Song Y, Esmon NL, Esmon CT. The Ser219->Gly dimorphism of the endothelial protein C receptor contributes to the higher soluble protein levels observed in individuals with the A3 haplotype. *J Thromb Haemost.* 2006;4(1):229-235.
 21. Saposnik B, Lesteven E, Lokajczyk A, Esmon CT, Aiach M, Gandrille S. Alternative mRNA is favored by the A3 haplotype of the EPCR gene PROCr and generates a novel soluble form of EPCR in plasma. *Blood.* 2008;111(7):3442-3451.
 22. National Center for Biotechnology Information. *dbSNP Short Genetic Variations Cluster Report: rs867186.* Available from: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?searchType=adhoc_search&type=rs&rs=rs867186. Accessed May 28, 2011.
 23. Rajeevan H, Cheung KH, Gadagkar R, et al. ALFRED: an allele frequency database for micro-evolutionary studies. *Evol Bioinform Online.* 2005;1:1-10.
 24. Smith NL, Chen MH, Dehghan A, et al. Novel associations of multiple genetic loci with plasma levels of factor VII, factor VIII, and von Willebrand factor: The CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) Consortium. *Circulation.* 2010;121(12):1382-1392.
 25. Reitsma PH, Rosendaal FR. Past and future of genetic research in thrombosis. *J Thromb Haemost.* 2007;5(suppl 1):264-269.
 26. Lowe GD. Common risk factors for both arterial and venous thrombosis. *Br J Haematol.* 2008;140(5):488-495.
 27. Prandoni P. Venous thromboembolism and atherosclerosis: is there a link? *J Thromb Haemost.* 2007;5(suppl 1):270-275.
 28. Ye Z, Liu EH, Higgins JP, et al. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. *Lancet.* 2006;367(9511):651-658.
 29. Kim RJ, Becker RC. Association between factor V Leiden, prothrombin G20210A, and methyl-ene-tetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: a meta-analysis of published studies. *Am Heart J.* 2003;146(6):948-957.
 30. Trégouët DA, Heath S, Saut N, et al. Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. *Blood.* 2009;113(21):5298-5303.
 31. Schunkert H, König IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet.* 2011;43(4):333-338.
 32. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. *Circulation.* 2008;117(1):93-102.
 33. Undas A, Szułdryński K, Brummel-Ziedins KE, Tracz W, Zmudka K, Mann KG. Systemic blood coagulation activation in acute coronary syndromes. *Blood.* 2009;113(9):2070-2078.
 34. Balding DJ. A tutorial on statistical methods for population association studies. *Nat Rev Genet.* 2006;7(10):781-791.
 35. Saposnik B, Remy J, Gaussem P, Emmerich J, Aiach M, Gandrille S. A haplotype of the EPCR gene is associated with increased plasma levels of sEPCR and is a candidate risk factor for thrombosis. *Blood.* 2004;103(4):1311-1318.
 36. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR, eds. *Introduction to Meta-Analysis.* Chichester, United Kingdom: John Wiley & Sons Ltd; 2009.
 37. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629-634.
 38. Ziegler A, Van Steen K, Wellek S. Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis. *Breast Cancer Res Treat.* 2011;128(1):197-201.
 39. Ioannidis JP, Boffetta P, Little J, et al. Assessment of cumulative evidence on genetic associations: interim guidelines. *Int J Epidemiol.* 2008;37(1):120-132.
 40. Little J, Higgins JPT. *The HuGENet HuGE Review Handbook.* Version 1.0. Available from: <http://www.hugenet.org.uk/resources/handbook.php>. Accessed February 28, 2006.
 41. Medina P, Navarro S, Estelles A, Vaya A, Bertina RM, Espana F. Influence of the 4600A/G and 4678G/C polymorphisms in the endothelial protein C receptor (EPCR) gene on the risk of venous thromboembolism in carriers of factors V Leiden. *Thromb Haemost.* 2005;94(2):389-394.
 42. Butterworth AS, Braund PS, Farrall M, et al. IBC 50K CAD Consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS Genet.* 2011;7(9):e1002260.
 43. Galanaud JP, Cochery-Nouvellon E, Alonso S, et al. Paternal endothelial protein C receptor 219Gly variant as a mild and limited risk factor for deep vein thrombosis during pregnancy. *J Thromb Haemost.* 2010;8(4):707-713.
 44. Bezemer ID, Bare LA, Doggen CJ, et al. Gene variants associated with deep vein thrombosis. *JAMA.* 2008;299(11):1306-14.
 45. Yamagishi K, Cushman M, Heckbert SR, Tsai MY, Folsom AR. Lack of association of soluble endothelial protein C receptor and PROCr 6936A/G polymorphism with the risk of venous thromboembolism in a prospective study. *Br J Haematol.* 2009;145(2):221-226.
 46. Pecheniuk NM, Elias DJ, Xu X, Griffin JH. Failure to validate association of gene polymorphisms in EPCR, PAR-1, FSAP and protein S Tokushima with venous thromboembolism among Californians of European ancestry. *Thromb Haemost.* 2008;99(2):453-455.
 47. Chen XD, Tian L, Li M, Jin W, Zhang HK, Zheng CF. Relationship between endothelial cell protein c receptor gene 6936a/g polymorphisms and deep venous thrombosis. *Chin Med J (Engl).* 2011;124(1):72-75.
 48. Medina P, Navarro S, Corral J, et al. Endothelial protein C receptor polymorphisms and risk of myocardial infarction. *Haematologica.* 2008;93(9):1358-1363.
 49. National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention. *Featured Data & Statistics: Prevalence of Heart Disease—United States (2005).* Available from: <http://www.cdc.gov/DataStatistics/archive/heartdisease.html>. Accessed April 18, 2011.
 50. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics.* 2003;19(1):149-150.
 51. Tang W, Basu S, Kong X, et al. Genome-wide association study identifies novel loci for plasma levels of protein C: the ARIC study. *Blood.* 2010;116(23):5032-5036.
 52. Rosendaal FR. Venous thrombosis: the role of genes, environment, and behavior. *Hematology Am Soc Hematol Educ Program.* 2005;2005:1-12.
 53. Guella I, Duga S, Ardissino D, et al. Common variants in the haemostatic gene pathway contribute to risk of early-onset myocardial infarction in the Italian population. *Thromb Haemost.* 2011;106(4):655-664.