

Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66 155 cases and 91 307 controls



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Summary

Background Variants of certain haemostatic genes (such as that encoding factor V Leiden) are involved in the development of venous thrombosis, but studies of such variants in coronary disease have reported apparently conflicting results. We did meta-analyses on seven such haemostatic genetic variants for which the available evidence on each comprises at least 5000 coronary disease cases and at least 5000 controls.

Methods Meta-analyses were done of 191 studies in relation to factor V G1691A (ie, factor V Leiden), factor VII G10976A, prothrombin G20210A, plasminogen activator inhibitor-1 (PAI-1) [-675] 4G/5G, and three platelet glycoprotein (GP) receptor variants (GPIa C807T, GPIb α T[-5]C, GPIIIa C1565T), involving a total of 66 155 coronary disease cases and 91 307 controls. We explored potential sources of heterogeneity.

Findings In a combined analysis of all studies, the per-allele relative risks (RR) for coronary disease of factor V 1691A and of prothrombin 20210A were 1.17 (95% CI 1.08–1.28) and 1.31 (1.12–1.52), respectively. Combined analyses of studies of the PAI-1 [-675] 4G variant yielded a per-allele relative risk for coronary disease of 1.06 (1.02–1.10), but there was an indication of publication bias in these studies. Combined analyses of the factor VII 10976A, GPIa 807T, GPIb α [-5]C, and GPIIIa 1565T variants showed no significant overall associations with coronary disease, yielding per-allele RRs of 0.97 (0.91–1.04), 1.02 (0.97–1.08), 1.05 (0.96–1.13), and 1.03 (0.98–1.07), respectively.

Interpretation The 1691A variant of the factor V gene and the 20210A variant of the prothrombin gene, both of which increase circulating thrombin generation, might each be moderately associated with the risk of coronary disease. Further studies are merited to assess these associations in greater detail (including any gene–gene and gene–environment interactions) and to determine any implications with regard to potential therapies designed to reverse patients' prothrombotic phenotype, such as selective plasma factor V or factor Xa inhibition.

Introduction

Certain genetic variants associated with abnormal haemostasis substantially increase the risk of venous thromboembolism in carriers because of defined biochemical alterations caused by the polymorphisms. For example, the G1691A polymorphism of the factor V gene (ie, factor V Leiden) enhances activated protein C resistance, and is associated with an approximately three-fold increase in risk of venous thromboembolism in heterozygotes and a greater than ten-fold increase in risk in homozygotes.^{1–3} By contrast, investigations of such haemostatic gene variants in arterial disease, most notably coronary disease, have generally indicated much weaker associations in a large number of apparently conflicting and inconclusive reports.^{2–7} Most such studies of coronary disease have not involved more than a few hundred disease cases, which is too few to assess reliably any genetic effects that might be realistically expected (such as per-allele relative risk [RR] increases of about 10–20%), particularly for the investigation of fairly uncommon variants (eg, the prevalence of factor V Leiden is only about 3% in white populations^{2–7}). Furthermore, the interpretation of available studies has been complicated by the use of different coronary disease endpoints (eg, myocardial infarction and coronary stenosis), different populations (European

continental ancestry vs other ethnicities), different population sampling strategies, and different genotyping procedures.

We have identified variants in seven genes related to haemostasis for which the available evidence on each polymorphism comprises, in aggregate, at least 5000 coronary disease cases and at least 5000 controls (webappendix, references 1–166). Each of these polymorphisms alters the function or plasma level of an intermediate phenotype involved in a haemostatic pathway,^{8–14} including coagulation (factor V G1691A, factor VII G10976A, prothrombin G20210A), fibrinolysis (plasminogen activator inhibitor-1 [PAI-1] [-675] 4G/5G), and platelet glycoprotein (GP) receptor function (GPIa C807T, GPIb α T[-5]C, GPIIIa C1565T) (table 1). We report new meta-analyses of studies of two of these variants (ie, GPIa C807T and GPIb α T[-5]C) as well as updated meta-analyses of the remaining five variants.^{15–26}

Methods

Relevant genes

To allow reliable assessment of the relevance of haemostatic gene polymorphisms to coronary disease, the present review focuses on seven relevant polymorphisms for which the available evidence comprises, in aggregate, at least 5000 cases and at least

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See Online for webappendix

	Main effect of intermediate phenotype	Chromosome location of gene	Length of gene (kb)	Number of exons	Position of polymorphism	Polymorphisms/rs number	Effects of polymorphism on intermediate phenotype	Approximate frequencies of rare allele in white people/Asians/African-Americans*
Coagulation								
Factor V	Cofactor in conversion of prothrombin to thrombin by factor Xa	1q23	72-31	25	Exon 10	G1691A (Arg506Gln or R506Q)/rs6025	Increases activated protein C resistance	3%/0.2%/?
Factor VII	Initiates coagulation in association with tissue factor	13q34	14-24	8	Exon 8	G10976A (Arg353Gln or R353Q)/rs6046	Decreases plasma factor VII levels by about 20%	13%/6%/?
Prothrombin (factor II)	Precursor of thrombin	11p11-q12	11-51	14	3' untranslated region	G20210A/NA	Raises plasma prothrombin levels by about 20%	1%/0.5%/?
Fibrinolysis								
PAI-1	Fast-acting inhibitor of tissue-type plasminogen activator	7q21.3-q22.1	11-86	9	Promoter	(-675) 4G/5G/NA	Raises plasma PAI-1 levels by about 20%	49%/46%/?
Platelet receptors								
GPIa	Receptor for collagen	5q23-q31	102-93	30	Exon 7	C807T/rs1126643	Might increase receptor density	38%/36%/?
GPIb α	Receptor for von Willebrand factor and thrombin	17pter-p12	2-57	2	Promoter	T(-5)/rs2243093	Might increase expression of the GPIb-IX-V complex by about 50%	13%/17%/?
GPIIIa	Receptor for fibrinogen (at low shear rates) and von Willebrand factor (at high shear rates) and other ligands	17q21.32	57-97	15	Exon 2	C1565T (Leu33Pro or L33P)/rs5918	Might increase sensitivity to platelet aggregation	15%/0.5%/12%

*Frequency of rare allele based on data derived from control groups in this meta-analysis. NA=not available. ?=unknown. Rs number: RefSNP accession identification number in SNP database.

Table 1: Description of haemostatic gene polymorphisms in this review and their associated intermediate phenotypes

5000 controls (table 1). Two further potentially eligible polymorphisms (both located in the promoter region of the β -fibrinogen gene) have not been included in the present meta-analyses because they have been reviewed recently elsewhere (Keavney BD and colleagues, unpublished data).²⁷ The following seven haemostatic gene polymorphisms are the focus of the present review: the G→A substitution at position 1691 of the factor V gene, resulting in an arginine to glutamine exchange in codon 506 (commonly referred to as Arg506Gln, factor V Leiden, or R506Q);² the G→A exchange at position 10976 in the factor VII gene, which results in an arginine to glutamine exchange in codon 353 (also known as Arg353Gln or R353Q); the G→A exchange at position 20210 in the 3' untranslated region of the prothrombin gene; the 4G/5G insertion/deletion in the PAI-1 gene at a position -675 of the promoter region; the C→T substitution at position 807 in the GPIa gene; a C→T substitution at position 1565 in exon 2 of the GPIIIa gene, which results in a leucine to proline exchange in codon 33 (Leu33Pro or PI^{A1/A2}); and the T→C substitution recently identified at position -5 upstream of the ATG initiation codon in the GPIb α gene (the von Willebrand factor-binding subunit of the complex). These polymorphisms occur in frequencies consistent with Hardy-Weinberg equilibrium in the control populations of the large majority of available studies (see webtable). Because the majority of the studies in the present meta-analyses reported on only one polymorphism, analyses of haplotypes (ie, combinations

of alleles at multiple positions along a genomic segment of a single chromosome) cannot be provided.

Search strategy and selection criteria

Epidemiological genetic association studies published before January, 2005, on coronary disease and at least one of the seven polymorphisms in the haemostatic genes described above were sought by computer-based searches, scanning of the reference lists of articles identified for all relevant studies and review articles (including meta-analyses), hand-searching of relevant journals, and correspondence with authors of included studies. Computer searches of PubMed, Web of Science, and EMBASE used keywords relating to the relevant genes (eg, "factor V", "factor VII", "prothrombin", "factor II", "plasminogen activator inhibitor", "PAI", "GPIa", "GPIb α ", "GPIIIa") in combination with words related to coronary heart disease (eg, "coronary heart disease", "myocardial infarction", "atherosclerosis", "arteriosclerosis", and "coronary stenosis") and polymorphism without language restriction. All relevant studies identified were included apart from six in which genotype frequencies were unavailable even after several rounds of correspondence (webappendix, references 167–172 and 197). 21 studies were excluded because controls were reported to have been selected on the basis of a positive diagnosis of coronary disease or angina (webappendix, references 173–193) and a further three studies were excluded because their data duplicated or overlapped, or

See Online for webtable

both, with reports already included in the review (webappendix, references 194–196).

Data abstraction

The following information was abstracted from each study, according to a fixed protocol: study design, geographical location, ethnic group of participants, definition and numbers of cases and controls, DNA extraction and genotyping methods, blinding of genotyping staff to case-control status of samples, frequency of genotypes, mean age of cases, and proportion of cases who were male (see webtable). Confirmation of genotype frequencies and genotyping procedures was also sought by correspondence with investigators (103 of 191 replied). Previously unreported data on other polymorphisms described in this review were included from 23 of these studies after such correspondence. In the few instances in which genotype frequencies provided by the investigators in tabular data differed slightly from published figures, the tabular data were used. Relevant clinical outcomes included confirmed myocardial infarction (generally by WHO criteria) and coronary stenosis (defined variously as at least 50% [webappendix, references 9, 15, 20, 52, 54, 60, 70, 98, 107, 136, 139, 162, 166] or 70% [webappendix, references 1, 30, 104, 150] stenosis of one or more major coronary arteries on the basis of computer-assisted assessments). For studies in which data could not be separated according to type of coronary disease from published data, even after correspondence (webappendix, references 67, 82, 90, 102, 146) cases were classified in the more inclusive category of coronary stenosis for the purpose of subsidiary analyses. Studies with different ethnic groups were considered as individual studies for our analyses (webappendix, reference 84).

Statistical analysis

The per-allele odds ratio (relative risk) of the rare allele (1691A, 10976A, 20210A, [-675]4G, 807T, [-5]C, 1565T) was compared between cases and controls by assigning

scores of 0, 1, and 2 to homozygotes for the common allele, heterozygotes, and homozygotes for the rare allele, respectively, and calculating odds ratios per unit score by logistic regression; this method is analogous to modelling a co-dominant model of inheritance. Fixed-effect summary measures were calculated as the inverse-variance weighted average of the log odds ratios. Subsidiary analyses involved use of a random-effects model and of dominant and recessive genetic models, where relevant. To make some allowance for multiple comparisons, 99% CI were used for individual studies, and 95% CI were reserved for the combined estimates. Heterogeneity was assessed using the I^2 statistic, which describes the percentage of variation in the log odds ratios that might be attributable to genuine differences across studies rather than to random error,²⁸ a statistical test,²⁹ and by using random-effects regression models with restricted maximum likelihood estimation. Study size, genotyping procedures, source of controls, ethnicity, and geographical location were prespecified as characteristics for assessment of heterogeneity; other potentially relevant subgroups (such as by age and haplotypes) could not be reliably investigated, since individual participant data were not available for this meta-analysis. Ethnic group was defined as Caucasian (ie, people of European continental ancestry), East Asian, African-American, or other (eg, Turks and Lebanese were not included in the Caucasian category). Statistical analyses were done with Stata (version 8.0) statistical software. In the figures, the area of the squares for individual studies or sets of studies (and of filled circles for overall results) are inversely proportional to the variances of the log odds ratio estimates, and horizontal lines indicate CIs (in the webfigures, diamonds denote pooled estimates, the width of which represents the CIs).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or

	Studies of myocardial infarction and coronary stenosis combined			I^2	Myocardial infarction			I^2	Coronary stenosis			I^2
	Number of studies	Number of cases/controls	Per-allele RR (95% CI)		Number of studies	Number of cases/controls	Per-allele RR (95% CI)		Number of studies	Number of cases/controls	Per-allele RR (95% CI)	
Factor V, G1691A	60	15 704/26 686	1.17 (1.08–1.28)	17%	53	12 518/23 374	1.22 (1.10–1.35)	28%*	15	3186/14 071	1.06 (0.89–1.25)	0%
Factor VII, G10976A	24	7444/12 110	0.97 (0.91–1.04)	0%	21	5286/8578	0.95 (0.88–1.03)	12%	10	2158/6754	1.01 (0.90–1.14)	0%
Prothrombin (factor II), G20210A	40	11 625/14 462	1.31 (1.12–1.52)	27%	30	8211/12 356	1.25 (1.05–1.50)	45%*	15	3414/3663	1.46 (1.10–1.94)	0%
PAI-1, [-675]4G/5G	37	11 763/13 905	1.06 (1.02–1.10)	57%‡	31	9143/12 793	1.04 (1.00–1.09)	52%†	11	2620/3217	1.12 (1.04–1.21)	64%*
GP1a, C807T	15	6414/7732	1.02 (0.97–1.08)	37%	13	4579/5485	1.02 (0.96–1.08)	28%	5	1835/2985	1.04 (0.94–1.16)	61%*
GP1bα, T[-5]C	14	6652/5188	1.05 (0.96–1.13)	36%	9	28 10/2959	1.03 (0.92–1.16)	49%*	5	3842/2752	1.05 (0.94–1.19)	14%
GP1IIa, C1565T	43	16 984/22 893	1.03 (0.98–1.07)	38%†	38	10 171/20 459	1.02 (0.96–1.07)	41%*	18	6812/5929	1.04 (0.97–1.13)	34%*
Total	191	66 155/91 307				42 054/72 985				24 101/37 402		

Heterogeneity p value: *p<0.01, †p<0.001, ‡p<0.0001. I^2 =measure of extent to which between-study variation is not due to chance alone, on a scale from 0% to 100%.

Table 2: Meta-analyses of 191 studies of seven haemostatic gene polymorphisms in 66 155 cases of coronary disease and 91 307 controls, analysed with a co-dominant genetic model

writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

A total of 191 relevant genetic association studies (168 published and 23 unpublished) were identified, with 33 studies genotyping more than one variant (table 2). A list of the relevant details abstracted from these studies is provided in the webtable. The total includes 60 studies (from 51 published studies [two of which involved two separate studies each (webappendix, references 84, 88)] and seven unpublished studies) of the G1691A polymorphism, 24 studies (including two unpublished studies) of the G10976A polymorphism, 40 studies (including four unpublished studies) of the G20210A polymorphism, 37 studies (including four unpublished reports) of the -675 4G/5G polymorphism, 15 studies (included one unpublished study) of the C807T polymorphism, 14 studies (including three unpublished studies) of the T[-5]C polymorphism, and 43 studies (including two unpublished studies) of the C1565T polymorphism. Studies were undertaken in a

wide range of geographical settings, with 68% (45 180 of 66 155) of cases being Caucasian, 11% (7321 of 66 155) Asian, and 21% (13 684 of 66 155) of other ethnic origins (including African-American). Of the 191 studies, 176 involved retrospective comparisons and 15 were prospective in design (involving internal controls; webappendix, references 16, 24, 37, 42, 59, 75, 88, 100, 128–132, 144, 152). Of the 176 retrospective studies, 79 drew controls randomly from approximately general populations, 16 involved controls from screening, blood-donor, or health-check visits, 34 recruited controls from outpatients or other patient groups, and six involved controls identified following negative cardiac assessment (webappendix, references 60, 109–112, 139). 14 of 176 retrospective studies (webappendix, references 5, 47, 48, 55, 74, 86, 97, 101, 121, 133, 135, 145, 149, 152) matched controls to cases by age, and a further 49 matched controls to cases by age and at least one other risk factor. All but 13 studies (webappendix, references 7, 39, 50, 56, 73, 81, 114, 120, 132, 147, 150, 159, 160) used PCR/restriction fragment length polymorphism (RFLP) with various restriction enzymes. Of the remaining studies, 12 used oligonucleotide probes

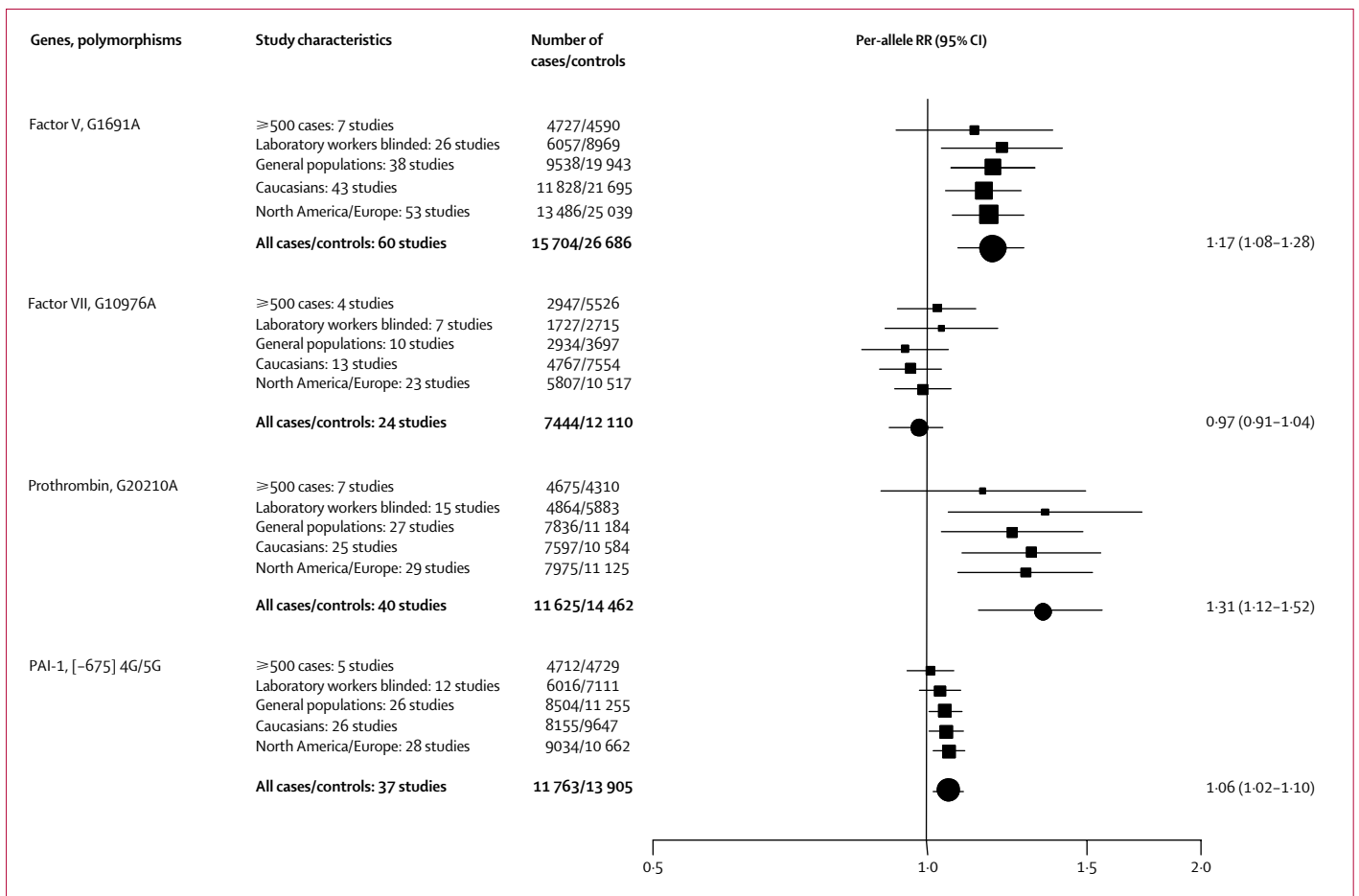


Figure 1: Meta-analyses of studies of coronary diseases and four haemostatic gene polymorphisms, grouped by various characteristics

(webappendix, references 7, 39, 50, 56, 73, 81, 114, 120, 132, 147, 159, 160) and one used denaturing high-performance liquid chromatography (DHPLC) and variant array (webappendix, reference 150).

Polymorphisms in coagulation or fibrinolytic genes

There was little heterogeneity among the 60 available studies (a total of 15 704 cases and 26 686 controls) of the 1691A variant in the factor V gene and total coronary heart disease ($I^2=17\%$, 95% CI 0–38; $p=0.12$), with no substantial variations when studies were grouped by the characteristics described in figure 1. Overall, the per-allele RR of the 1691A variant for total coronary heart disease was 1.17 (1.08–1.28; figure 1 and webfigure 1), with an RR of 1.17 (1.07–1.28) under a dominant genetic model (the very low prevalence of the AA genotype prevented calculation of the RR under a recessive model). Subsidiary analyses of specific coronary heart disease endpoints yielded a per-allele RR for myocardial infarction of the 1691A variant of 1.22 (1.10–1.35) and for coronary stenosis of 1.06 (0.89–1.25), with no clear difference between these subtotals ($\chi^2=3.15$; $p=0.08$). There was no evidence of heterogeneity among the 24 available studies (a total of 7444 cases and 12 110 controls) of the 10976A variant in the factor VII gene and total coronary heart disease ($I^2=0\%$, 0–40; $p=0.55$). Overall, the per-allele RR of the 10976A variant for total coronary heart disease was 0.97 (0.91–1.04; figure 1 and webfigure 2), with corresponding results under dominant and recessive genetic models of 0.98 (0.91–1.05) and 0.94 (0.81–1.10), respectively. There was marginal evidence for a moderate degree of heterogeneity among the 40 available studies (including a total of 11 625 cases and 14 462 controls) of the 20210A variant in the prothrombin gene and total coronary heart disease ($I^2=27\%$, 0–49; $p=0.05$), but little of it was explained by the factors described in figure 1. Overall, the per-allele RR of the 20210A variant for total coronary heart disease was 1.31 (1.12–1.52; figure 1 and webfigure 3), with an RR of 1.33 (1.14–1.55) under a dominant genetic model (again, the very low prevalence of the GG genotype prevented calculation of the RR under a recessive model). There was substantial heterogeneity among the 37 available studies (a total of 11 763 cases and 13 905 controls) of the [–675] 4G variant in the PAI-1 gene and total coronary heart disease ($I^2=57\%$, 39–69; $p<0.0001$), but little of it was explained by the factors described in figure 1. Overall, the per-allele RR of the [–675] 4G variant for total coronary heart disease was 1.06 (1.02–1.10; figure 1 and webfigure 4), with corresponding results under dominant and recessive genetic models of 1.07 (1.00–1.14) and 1.07 (1.01–1.13), respectively. Subsidiary analyses of specific coronary heart disease endpoints yielded a per-allele RR for myocardial infarction of the [–675] 4G variant of 1.04 (1.00–1.09)

and for coronary stenosis of 1.12 (1.04–1.21), with no clear difference between these subtotals ($\chi^2=2.66$; $p=0.10$). A funnel plot suggested evidence of more strikingly positive findings in the smaller published studies of the [–675] 4G variant (Begg's test, $p=0.003$; webfigure 5), but such plots were not similarly suggestive for the six other variants reviewed (data available on request).

Polymorphisms in platelet receptor glycoprotein genes

There was marginal evidence of heterogeneity among the 15 available studies (a total of 6414 cases and 7732 controls) of the 807T variant in the GPIa gene and total coronary heart disease ($I^2=37\%$, 0–65; $p=0.06$). Overall, the per-allele RR of the 807T variant for total coronary heart disease was 1.02 (0.97–1.08; figure 2 and webfigure 6), with corresponding results under dominant and recessive genetic models of 1.02 (0.95–1.09) and 1.05 (0.95–1.10), respectively. Similarly, there was marginal evidence of some heterogeneity among the 14 available studies (a total of 6652 cases and 5188 controls) of the [–5]C variant in the GPIb α gene and total coronary heart disease ($I^2=36\%$, 0–66; $p=0.09$). Overall, the per-allele RR of the [–5]C variant for total coronary heart disease was 1.05 (0.96–1.13; figure 2 and webfigure 7), with corresponding results under dominant and recessive genetic models of 1.04 (0.95–1.14) and 1.17 (0.87–1.58), respectively. There was moderate heterogeneity among the 43 available studies (a total of 16 984 cases and 22 893 controls) of the 1565T variant in the GPIIb gene and total coronary heart disease ($I^2=38\%$, 16–54; $p=0.001$), but little of this was explained by the factors described in figure 2. Overall, the per-allele RR of the 1565T variant for total coronary heart disease was 1.03 (0.98–1.07; figure 2 and webfigure 8), with corresponding results under dominant and recessive genetic models of 1.03 (0.98–1.08) and 1.05 (0.90–1.22), respectively.

Discussion

The present meta-analysis of 191 studies, involving a total of 66 155 cases and 91 307 controls (counting every study's cases and controls only once), provides the most comprehensive assessment so far of the relevance to coronary disease of seven haemostatic gene polymorphisms. It provides the first clear indication of moderate and highly significant associations of coronary disease risk with the 1691A variant of the factor V gene (ie, factor V Leiden) and with the 20210A variant of the prothrombin gene, both of which increase circulating thrombin generation.³⁰ The findings of the meta-analysis also indicate a weakly positive association of the [–675] 4G variant of the PAI-1 gene with the risk of coronary disease; although, as discussed below, this finding might be mainly an artefact of selective publication. By contrast with previous suggestions based

See Online for webfigures 1–8

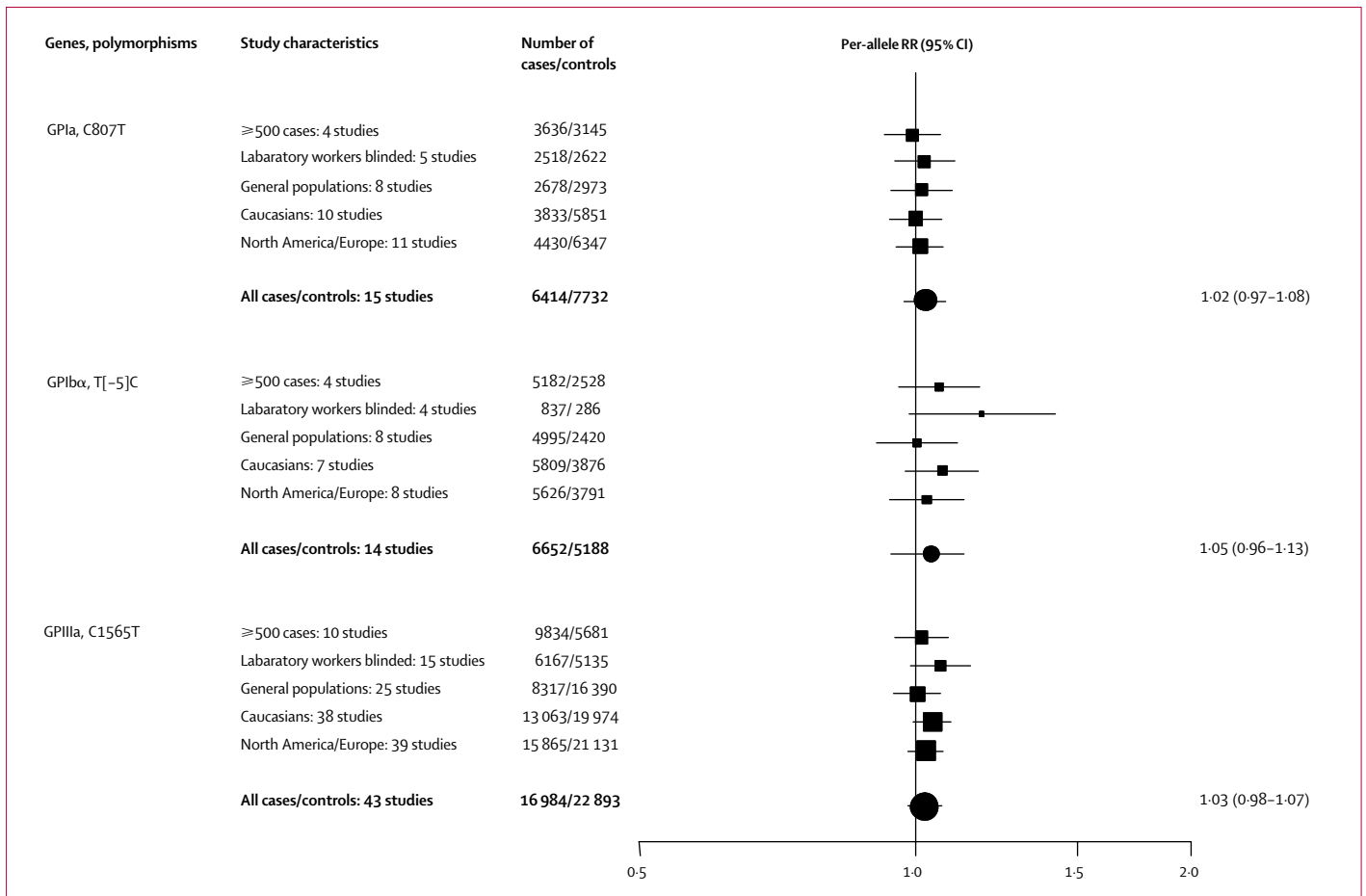


Figure 2: Meta-analyses of studies of coronary diseases and three platelet receptor polymorphisms, grouped by various characteristics

on substantially less data,^{15,16} the present report has shown essentially null associations of coronary disease with the 10976A variant of the factor VII gene and with three platelet glycoprotein receptor polymorphisms (ie, 807T, [-5]C, and 1565T of the GPIa, Ib α , and IIIa genes, respectively).

The findings of the present meta-analysis of studies of factor V Leiden, which involves about five times as much data as in a previous review that reported a non-significant result,¹⁸ indicate a highly significant per-allele relative risk for coronary disease (table 3). Homozygosity for factor V Leiden in mice can lead to enhanced arterial thrombosis and atherosclerosis, apparently mediated by circulating, non-platelet-derived factor V Leiden.³¹ However, although such findings are consistent with a causal interpretation of the epidemiological data for coronary disease, further investigation of factor V Leiden is needed, either by fresh genotyping in large-scale studies or by more detailed pooling of available data (or both), in order to assess age-specific and sex-specific associations, any gene–gene interactions, and gene–environment interactions (in particular, any synergy with cigarette smoking, levels of blood lipids, or

oral oestrogen use³²). Similar considerations apply to the 20210A polymorphism of the prothrombin gene, for which the findings of the present meta-analysis, involving about three times as much data as in a previous review that reported a non-significant result,²² indicate a highly significant per-allele relative risk for coronary disease (table 3). Although the scope for publication bias in the present review has been reduced substantially by attempts to identify unreported data by correspondence with investigators (and is not suggested by funnel plots, and other relevant tests for the available studies of these two variants), the possibility of such residual biases is difficult to exclude in meta-analyses of the published work.³³ Future genotyping efforts that focus on very large individual studies (or on predefined consortia of several studies) should help in this regard, particularly because data from studies with more than 500 cases (which should be less prone to publication bias than are smaller studies) are still comparatively limited for these two variants (table 3).

Results of a previous meta-analysis suggested a strong inverse association between the G10976A variant in the factor VII gene and the risk of coronary disease (table 3).¹⁶

	Previous meta-analyses (most recent)	Present meta-analyses	
		All studies	Studies with ≥ 500 cases
Factor V, G1691A	1.18 (0.97–1.43)* 19 studies: 3270 cases, 5459 controls ¹⁶	1.17 (1.08–1.28)* 60 studies: 15 704 cases, 26 686 controls	1.12 (0.91–1.36)* 7 studies: 4727 cases, 4590 controls
Factor VIII, G10976A	0.53 (0.27–1.03)† 6 studies: 1258 cases, 1316 controls*	0.97 (0.91–1.04)† 24 studies: 7444 cases, 12 110 controls	1.03 (0.93–1.13)† 4 studies: 2947 cases, 5526 controls
Prothrombin (factor II), G20210A	1.24 (0.98–1.63)* 15 studies: 3965 cases, 6275 controls ¹⁵	1.31 (1.12–1.52)* 40 studies: 11 625 cases, 14 462 controls	1.19 (0.91–1.55)* 7 studies: 4675 cases, 4310 controls
PAI-1, [-675] 4G/5G	1.20 (1.04–1.39)† 10 studies: 1515 cases, 1866 controls ¹³	1.06 (1.02–1.10)† 37 studies: 11 763 cases, 13 905 controls	1.01 (0.95–1.07)† 5 studies: 4712 cases, 4729 controls
GP1a, C807T	NA	1.02 (0.97–1.08)† 15 studies: 6414 cases, 7732 controls	0.99 (0.92–1.07)† 4 studies: 3636 cases, 3145 controls
GP1b α , T[-5]C	NA	1.05 (0.96–1.13)† 14 studies: 6652 cases, 5188 controls	1.06 (0.96–1.18)† 4 studies: 5182 cases, 2528 controls
GP11a, C1565T	1.10 (0.95–1.26)† 19 studies: 5298 cases, 5285 controls ¹⁴	1.03 (0.98–1.07)† 43 studies: 16 984 cases, 22 893 controls	1.02 (0.95–1.09)† 10 studies: 9834 cases, 5681 controls

*Dominant model. †Co-dominant model. NA=not available.

Table 3: Comparison of findings of present meta-analyses of 191 studies (including 66 155 cases of coronary disease and 91 307 controls) with those reported in previous meta-analyses of same polymorphisms in seven haemostatic genes, using the same genetic models as in earlier analyses

The present meta-analysis, which involves about eight times more data than in the earlier review, refutes this claim (table 3). Similarly, by contrast with a previous review of studies that suggested a per-allele relative risk of about 1.2 for coronary disease in people with the [-675] 4G variant of the PAI-1 gene (table 3),¹⁵ the present meta-analysis yielded a per-allele relative risk of just 1.06 (1.02–1.10) based on about ten times more data. Moreover, given the indication of publication bias in the funnel plot of studies of this variant, the weaker non-significant combined relative risk of only 1.01 (0.95–1.07) yielded from the five studies with at least 500 cases might be a better estimate. As regards the platelet receptor polymorphisms considered in glycoprotein genes Ia, Ib α , and IIIa, the per-allele relative risks for each were essentially null, with fairly narrow confidence limits. But despite the lack of important associations between the risk of coronary disease and these five polymorphisms, the present data do not necessarily exclude the possibility that other variants (or combinations of alleles at multiple positions) in the same genes could be materially relevant to coronary disease, particularly given that these genes are relatively large (table 1). Hence, the present data should not discourage the use of more recently employed approaches—such as resequencing complete genomic regions of candidate genes to identify potentially relevant variants (including haplotypes) and whole-genome association studies, involving hundreds of thousands of anonymous markers—that might extend evidence on these haemostatic genes beyond that provided by the candidate variant approach used in the available studies.³⁴

The present report suggests two major conclusions. First, specific variants in the factor V and prothrombin genes, both of which are important in pathways that increase circulating thrombin generation, might each be associated with moderate increases in the risk of

coronary disease. These findings could have implications for designing therapies to reverse the prothrombotic phenotype of patients—for example, it might be possible to reduce relative thrombophilia due to the factor V Leiden mutation by selectively targeting plasma factor V (or factor Xa, for which factor V is a co-factor in conversion of prothrombin to thrombin) while maintaining physiological haemostasis.³⁵ Second, and more generally, the present findings reinforce the need to undertake much larger studies (including updated meta-analyses) than have been customary in genetic epidemiology in order to assess sufficiently reliably any modest (but still potentially important) effects that might realistically be expected in complex conditions such as coronary disease.^{36–38} The present meta-analysis shows how such approaches could reduce to a minimum the likelihood both of false-negative and of false-positive results, thereby helping to identify promising hypotheses for further investigation.

Contributors

J Danesh conceived and supervised the study, interpreted results, and drafted the manuscript. Z Ye conducted searches, abstracted data, corresponded with authors, analysed and interpreted results, and drafted the manuscript. E H C Liu conducted searches, abstracted data, provided epidemiological expertise, and edited the manuscript. J P T Higgins advised on statistical analyses, interpreted results, and edited the manuscript. B D Keavney advised on genetic analyses, interpreted results, and edited the manuscript. G D O Lowe advised on haemostasis, interpreted results, and edited the manuscript. R Collins advised on epidemiology, interpreted results, and edited the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

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