Correlation of Polymorphisms to Coagulation and Biochemical Risk Factors for Cardiovascular Diseases

Alan H.B. Wu, PhD, and Gregory J. Tsongalis, PhD

Currently, the established risk factors for cardiovascular disease (CVD) are largely environmental in nature. Conflicting studies have suggested that mutations in specific coagulation genes may also provide a genetic basis for CVD risk. We reviewed clinical studies that examined the role of single nucleotide polymorphisms in coagulation and platelet factors, and a biochemical factor to determine if specific genotypes are correlated with patients with a history of arterial thrombotic diseases (acute coronary syndromes or stroke). A meta-analysis was performed on studies for factors II (G20210A variant), V Leiden (G1691A), VII (R353Q), glycoprotein (GP) IIIa receptor (PIA1/A2), and methylenetetrahydrofolate reductase (MTHFR, C677T). There was no correlation for factor II or factor V polymorphisms to coronary artery disease (CAD) in 5,607 and 5,431 patients studied, respectively. There was also no correlation for factor II variants and stroke in 3,451 patients studied. For factor V, statistical significance was achieved for the G1691A variant on 3,399 patients with stroke (odds ratio [OR] 1.43, 95% confidence intervals [CI] 1.03 to 1.97). The GP IIIa PIA1/A2 genotype was associated with increased risk for CAD in 7,920 patients (OR 1.12, 95% CI 1.01 to 1.24), but not for 1,855 patients who had a stroke (OR 0.80, 95% CI 0.62 to 1.04). The combined RQ and RR genotypes of factor VII R353Q were correlated to a reduced risk for CVD in 2,574 patients (OR 0.78, 95% CI 0.65 to 0.93), whereas the QQ genotype had offered more protection (OR 0.53, 95% CI 0.27 to 1.03). The TT homozygous variant of MTHFR was associated with CAD risk in 5,644 patients studied (OR 1.30, 95% CI 1.11 to 1.52) but not for 3,075 patients with stroke. This study shows that for some genes, further studies are unnecessary, whereas for others, no more enrollments are needed. The impact of certain genotypes must be examined in relation to other established risk factors and potentially new therapeutic strategies.

METHODS

A Medline literature search was conducted using the search terms “polymorphism” combined with “factors II, V Leiden, VII, GP IIIa, and MTHFR.” The search was conducted for studies written in English from 1993 to 2000. Only articles that correlated single nucleotide polymorphisms (SNPs) of these coagulation factors with arterial thrombotic diseases were considered. Data correlating SNPs to venous thrombotic diseases (deep vein thrombosis, pulmonary emboli, and so forth) were excluded. For studies that correlated venous and arterial thromboses, only data from arterial diseases were used.

For each study, a logistics analysis was conducted (Crunch v. 4, Oakland, California) to determine the OR (and 95% CI) of specific SNPs in patients with confirmed CVD against a cohort of age-matched control subjects without coronary artery disease (CAD).
A limitation of this study is that some of these control subjects may have had asymptomatic CVD, because they were selected on the basis of clinical history without necessarily having angiographic evidence of normal coronary and carotid arteries. In most published studies, the homozygous wild type was compared against the heterozygous variant of the gene. For all genes except MTHFR, the few homozygous variants that were found in the study population were combined into the heterozygous variant group. For MTHFR, the homozygous TT genotype was evaluated against the combined homozygous wild type and heterozygous variant polymorphisms. Patients with a history of unstable angina, acute myocardial infarction, or confirmed CVD were combined. Separate meta-analyses were conducted on patients with stroke or carotid atherosclerosis, which were combined. For factor II, the meta-analysis consisted of 8 studies on 5,607 patients with CAD and matched controls, and 5 studies on 3,451 patients with stroke and controls. For factor V Leiden, there were 9 studies on 5,431 patients with CAD and controls, and 6 studies on 3,399 stroke patients and controls. For factor VII, there were 6 studies on 2,574 patients and controls for CAD. There were insufficient numbers of studies published on the correlation of factor VII and stroke. For GP IIIa receptor, there were 10 studies on 7,920 patients and controls for CAD. For factor V Leiden and development of CVD (OR 1.24, 95% CI 0.96 to 1.60),7,10–12,16–20 For stroke,13,15,18,21–23 only 1 study alone demonstrated statistical significance for the G1691A variant.23 The cumulative meta-analysis achieved significance (OR 1.43, 95% CI 1.03 to 1.97, n = 3,399). This polymorphism warrants additional clinical studies in stroke patients.

One report each suggested that smokers with factor II and V Leiden mutations were at increased risk for CVD.5,19 These findings could not be confirmed by other studies, because logistics analysis using smoking status as a variable was not performed.

Each of the 6 studies1,11,12,24–27 on factor VII showed that the RQ or QQ genotypes were associated with a decreased incidence of CVD, although only 1 of these reached statistical significance.27 When combined, the OR was significant at 0.78 (95% CI 0.65 to 0.93, n = 2,574). The OR of <1.0 indicated that the variant RQ and QQ genotypes were associated with a lower incidence of CAD. When the QQ was compared with the RR genotype, the OR was even lower (OR 5.3, 95% CI 0.27 to 1.03, n = 1,885), although the ratio failed to reach statistical significance. These data suggested that the Q allele offered a protective effect toward development of CAD. The corollary of this conclusion is that patients with the RR genotype are associated with a higher incidence of CAD to the same degree that the RQ and QQ genotypes are protective.

Despite large trials suggesting the contrary, an unexpected finding was that there was statistical significance for GP IIIa receptor PI/A2 polymorphism and presence of CVD (OR 1.12, 95% CI 1.01 to 1.24, n = 7,920). The slight increase in the OR was achieved only when large numbers of studies were combined. There was no significance observed for stroke (OR 0.80, 95% CI 0.62 to 1.04, n = 1,885). For the TT variant of MTHFR, significance was achieved with CAD (OR 1.30, 95% CI 1.11 to 1.52, n = 5,644), whereas no significance was found in 3,075 patients with stroke.22,23,46–50

### RESULTS

The overall incidence of each variant allele was determined from the published data using the control populations. For factors II and V Leiden, the incidence of the A allele was 1.4% and 2.5%, respectively. There were no cases of the homozygous AA variant among the >3,000 control subjects studied for each gene. For factor VII, the Q allele frequency was 13.3%, with 1.6% of controls having the QQ homozygous genotype. For the GP IIIa receptor, the A2 allele frequency was 16.1% with 2.3% having the A2/A2 genotype. For the MTHFR gene, the T allele frequency was 43.3% with 13.1% having TT. Because most subjects in these studies were recruited from the United States and Western Europe, the Asian population was under-represented. Polymorphic patterns can differ between races; for example, factor V 1691A polymorphism is rare among Asians.4

Figures 1 to 5 illustrate the results of the meta-analysis, listed by publication date, along with the number of patients and controls enrolled, and study citation. For factor II and CVD,5–15 only 1 small study14 demonstrated a significant OR for the 20210A genotype and CVD. When combined with other studies, the OR was 1.15 for CVD (95% CI 0.84 to 1.59) and 1.35 (95% CI 0.89 to 2.05) for stroke,6,9,13–15 demonstrating no significant correlation for either disease. Figure 2 shows that there was no statistical significance for factor V Leiden and development of CVD (OR 1.24, 95% CI 0.96 to 1.60).7,10–12,16–20 For stroke,13,15,18,21–23 only 1 study alone demonstrated statistical significance for the G1691A variant.23 The cumulative meta-analysis achieved significance (OR 1.43, 95% CI 1.03 to 1.97, n = 3,399). This polymorphism warrants additional clinical studies in stroke patients.

### DISCUSSION

It has been known for many years that persons with a normal “lipid profile” (i.e., cholesterol, triglycerides, and high-density lipoprotein and low-density lipopro-
tein cholesterol) can still be at significant risk for future CVD. This knowledge has led to the search for other independent indicators of disease risk. Some of the more widely studied biochemical markers include lipoprotein (a), C-reactive protein, and oxidized low-density lipoprotein.

The general role of genetic factors was established by the National Cholesterol Education Program, when the presence of a family history of premature CVD death was listed as an independent risk factor. This has led to a search for specific gene variants that might be important adjuncts to biochemical screening tests. In the search for genetic cardiovascular markers, polymorphisms in genes that are involved in CVD pathogenesis are natural choices. If genetic risk factors for CVD can be identified, they have the inherent advantage over biochemical markers in that they are not influenced by diet, physiologic state, or medications.

The mechanism by which polymorphism in the factor VII gene affect CVD risk appears to be related to the correlation of these variants in the production of factor VII coagulant (FVIIc) and antigen (FVIIa) levels in blood. Bernardi et al showed that patients with the RR genotype had higher concentrations of these factors than those with the RQ genotype, which in turn were higher than in the QQ genotype. Blood concentration of these factors may determine the balance between the cardioprotective versus hypercoagulability state of the individual, with the R allele favoring the latter state.

The correlation of GP IIIa with a higher incidence of CAD relative to controls may have some impact on administering GP IIb/IIIa receptor inhibitors to patients with acute coronary syndromes, and those undergoing percutaneous coronary intervention. In angioplasty studies, patients with the PT allele, with and without stent placement, had a statistically higher restenosis rate at 6 months than patients with the wild type, suggesting that platelet inhibitors are less effective in these patients, or that higher dosages are needed. Clearly more studies are needed in this area.

MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, and is responsible for the remethylation of homocysteine to methionine. Mutation in the MTHFR gene is associated with increased plasma homocysteine, an established risk factor for CVD. This meta-analysis demonstrates a significant risk for the TT genotype relative to the wild type (3 studies alone reached significance). No individual or combined studies showed a correlation of the TT genotype with stroke.

Although some gene polymorphisms were linked to CVD and stroke, the ORs were very low, indicating that the risk was only slightly higher than a nondiseased population. A more meaningful genetic risk assessment may be to determine if the combination of several markers produces higher risk ratios. In addition to the genetic markers described in this analysis, there have been many other polymorphisms in genes encoding important biochemical or coagulation factors that have been studied, and may be candidates for...
a multivariate analysis (e.g., GP IIb, antiplatelet antigen-3 of GP IIb, apoproteins E and B-100, angiotensin-converting enzyme, low-density lipoprotein receptor, fibrinogen B, and factor VII HVR4 size polymorphism). The genetic markers studied in this report were selected because there are many published studies on them, because conclusions made by investigators were in conflict with each other, and the number of combined cases and control subjects were large enough so as to not limit the ability of a meta-analysis to arrive at a statistical conclusion. In the next few years, data on many other putative markers of CVD risk will become available warranting new meta-analyses. The development of novel technologies, such as DNA arrays will further hasten the research on genetic markers. The genetic makeup of patients with a family history and presence of confirmed CVD could be compared with those without a familial history or disease presence. Genotypic differences between these groups could be systematically explored. If a panel of genetic variants proves to be useful as an independent assessment for CVD risk, a micro-chip array may be an economical approach toward the use of these markers on a routine basis.

7. Doggen CJM, Cate VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular...


36. Jialal I, Devaraj S. Low-density lipoprotein oxidation, antioxidants, and PREVENTIVE CARDIOLOGY/CORRELATION OF POLYMORPHISMS TO CVD 1365

PREVENTIVE CARDIOLOGY/CORRELATION OF POLYMORPHISMS TO CVD


