Published in final edited form as:

Nutr Metab Cardiovasc Dis. 2011 August ; 21(8): 546–552. doi:10.1016/j.numecd.2009.12.002.

Polymorphisms in the platelet-specific collagen receptor GP6 are associated with risk of nonfatal myocardial infarction in Caucasians

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Abstract

Background and Aims—Glycoprotein 6 (GP6) is a platelet-specific collagen receptor implicated in the thrombotic pathway to acute myocardial infarction (AMI), but a possible genetic relationship between GP6 and AMI is poorly understood. We tested for the genetic association between AMI and single nucleotide polymorphisms (SNPs) in 24 loci, including GP6.

Methods and Results—We conducted a case-control study of AMI and GP6 in a community-based population (n=652 cases, 625 controls). We also examined men and women separately and stratified the latter by use of hormone replacement therapy (HRT). Among both sexes, the strongest association was for a protective missense polymorphism (rs1163662) in the GP6 gene (OR=0.70; Bonferroni-adjusted p<0.05). SNPs in GP6 were also strongly associated with AMI among women who reported ever taking HRT, but not among women who never took HRT. Haplotype analyses were consistent with the single-SNP findings.

Conclusions—In this sample of white non-Hispanic men and women, several SNPs in GP6 were significantly related to risk of AMI. Development of pharmacologic therapy directed towards platelet activity and thrombosis may reduce the incidence of AMI among at-risk groups.

Keywords

Acute myocardial infarction; glycoprotein 6; hormone replacement therapy

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Conflicts of Interest

All authors declare no conflicts of interest related to this work.

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Introduction

The processes underlying coronary atherosclerosis and acute myocardial infarction (AMI) are complex and multifactorial. Notably, disruption of unstable atheromatous plaques may lead to exposing thrombogenic material to blood under high shear conditions and the formation of coronary thrombosis (1–3). Of critical importance, the rupture of a lipid-laden, unstable plaque may stimulate thrombus formation in part by activating glycoprotein VI (GP6) (1), a platelet-specific collagen receptor from the immunoglobulin superfamily (4). Platelet activation and adhesion by components of the extracellular matrix play a critical role in formation of a coronary thrombosis, yet relatively little is known concerning the genetic contribution to thrombosis and AMI.

We tested for genetic association between nonfatal AMI and single nucleotide polymorphisms (SNPs) in 24 loci, including GP6, that have previously been related to thrombosis, inflammation or endothelial dysfunction, using data on individuals from the Western New York Study. Moreover, because estrogen is known to enhance the thrombotic potential of blood (5) and there is clinical trial evidence that HRT may be associated with early adverse effects and late benefit among women who have experienced an AMI (6), we also examined the effect of selected polymorphisms on AMI in postmenopausal women who reported use of hormone replacement therapy (HRT).

Materials and Methods

Study Population

The study design and methods used in the Western New York Study have been previously published (7,8). Briefly, incident cases of acute myocardial infarction (AMI) were recruited from hospitals in Western New York between 1996 and 2002. Individuals discharged alive with an AMI diagnosis (ICD 9 code 410–414, 425) between ages 35–69 and without a history of prior AMI, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, symptomatic angina pectoris, or diagnosis of other cardiovascular diseases were invited to participate. Hospital medical records were abstracted to allow diagnostic confirmation of AMI according to World Health Organization criteria (9). These procedures identified 95% of all AMIs in Erie and Niagara Counties. Among eligible cases, 59.5% agreed to participate in this investigation. This study was approved by the University's Health Sciences Institutional Review Board. Informed consent was obtained for all participants.

Control participants were randomly selected from the driver's license bureau rolls (for controls <65 years) and from the Health Care Financing Administration roster (for controls 65–75 years). For this report, we conducted a case-control study utilizing 926 cases and an equal number of controls that were individually matched on sex, ethnicity and age ± 1 year. Due to the hypothesis that HRT may influence risk of AMI, we analyzed men and women separately.

Measurements and Definitions

All participants completed a series of questionnaires and underwent a comprehensive interview and physical examination in our Center for Preventive Medicine. Cases were examined 4.4 months (± 1.3 mos) after the index AMI to minimize effects of the AMI on variables of interest. Controls were examined during the same time frame as the AMI patients. We collected data on sociodemographic characteristics, anthropometric variables including height and weight, personal and family medical history, medication use, alcohol use, smoking, and hormone replacement therapy (for women). Resting blood pressure (BP) was obtained according to established guidelines (10). Hypertension was defined as systolic

BP ≥ 140 mmHG, diastolic BP ≥ 90 mmHG, or history of using medications known to lower blood pressure. Type 2 diabetes was defined by (I) a positive answer to “has a physician ever told you that you have diabetes?” and concurrent use of insulin or oral antidiabetic medications, or (II) fasting blood glucose above 125 mg/dl for those without such a history. Total cholesterol was measured enzymatically (11) by CAP-certified Millard Fillmore Clinical Laboratory. Hyperlipidemia was defined as total cholesterol concentration ≥ 200 mg/dl or history of using lipid-lowering medication. For women, postmenopausal status was defined as a complete cessation of menses for the last 12 months or reported use of hormone replacement therapy. Obesity was defined as body mass index (BMI; calculated as weight in kg per squared height in cm) ≥ 30 kg/m². Family history of heart disease was defined as a positive self report of one or more first degree relatives with heart disease.

In the current report, we focused on associations between polymorphisms in candidate genes and AMI status, thus, to adjust for the effects of possible confounders we incorporated known risk factors for cardiovascular disease as covariates in our models. These covariates included: BMI (≥ 30 vs. < 30 kg/m²), hypertension (no/yes), diabetes (no/yes), hyperlipidemia (no/yes), smoking status (never/former/current), and positive family history of heart disease (no/yes).

Candidate Gene Selection and Genotyping

Twenty-four loci with known roles in biological pathways related to inflammation, collagen metabolism, and endothelial function were considered in this study. Initially, 33 single nucleotide polymorphisms (SNPs) with purported function or having exhibited evidence of association to CHD in previous studies were selected for genotyping. Based on our initial results of an observed association between AMI and a SNP in GP6 (rs1613662), 5 additional SNPs spanning the GP6 gene were also genotyped.

High molecular weight DNA was isolated from unanticoagulated whole blood by centrifugation of the clot through Clotspin tubes and DNA purification using the QIAamp DNA mini kit (QIAGEN Inc). SNPs were genotyped by the 5'-nuclease assay (12) using the ABI 7900HT (Foster City, CA) and the Taqman protocol and primers designed using the ABI Assays on Demand service. Individuals of known genotype were included in each run and clustering was analyzed using the V222 software. DNA samples were unavailable for 41 participants.

Quality Control

In order to obviate bias due to population stratification, nonwhite individuals were excluded from all analyses (n=114). We also excluded (1) individuals missing information on ≥ 1 of the covariates modeled in our analysis (n=262) (2) poor-quality DNA samples (i.e. missing genotype calls for > 4 of 38 genetic markers, n=158), and (3) genetic markers with low call rates (i.e. $> 10\%$ of genotypes not called). None of the SNPs differed significantly from HWE ($p > 0.01$ for all). After applying the quality control filters, genotype and phenotype data were available for 31 markers on 985 men (481 cases and 504 controls) and 292 women (144 cases and 148 controls) who comprised the analytical data set. Summary characteristics for the total analytic sample, men, women, cases, and controls are shown in Table 1.

Statistical Analysis

Analyses were performed in two stages: First, association between AMI and each of the 31 initial SNPs in 24 loci was tested using unconditional logistic regression, while simultaneously adjusting for the effects of the matching factors and the covariates. We assumed an additive genetic model for the effect of the SNP genotype. Analyses were performed over both sexes, and subsequently, for men and women separately. Loci

exhibiting evidence of association at $\alpha = 0.1$ after adjustment for multiple comparisons were genotyped for additional markers, and second stage analyses were performed on these. Stage 2 analyses included SNP-wise analyses using unconditional logistic regression and covariate adjustment (as in stage 1) as well as haplotype analyses using the haplo.stats package (13) to determine whether a specific haplotype was related to risk. Haplotypes, weighted by their estimated probabilities, were tested for association with AMI via logistic regression while simultaneously adjusting for covariates. The only locus meeting criteria for stage 2 analyses was GP6. Based on literature (14), we also tested for an interaction of GP6 with HRT in women, and performed stratified tests of genetic association at this locus (using unconditional logistic regression, as above, for both analyses).

We used two methods to control for the effect of testing multiple SNPs during stage 1: Bonferroni correction of asymptotic p-values across the 24 candidate loci, and assessment empirical p-values using 10 000 permutations. Empirical distributions were generated for the combined population, men, and women. Stage 2 analyses were not adjusted for multiple comparisons because these tests are locus-specific, extensively correlated, and designed to further explore the causes of the already-identified signal from stage 1. All analyses were conducted in the R Statistical Suite (R Foundation for Statistical Computing, Vienna).

Results

SNPs in candidate genes involved in inflammation, collagen content, and endothelial function were genotyped and tested for genetic association with AMI (Table 2) while simultaneously adjusting for age, BMI, smoking status, hypertension, diabetes, hyperlipidemia, and family history. The strongest observed genetic association with AMI in the total sample was for a missense polymorphism (rs1613662) in the GP6 gene (odds ratio [OR]=0.70, nominal $p=0.002$, empirical $p=0.068$, Bonferroni-adjusted $p=0.048$). Compared to the major allele, the minor allele ("C") of this SNP was protective against AMI, and the effect size was similar in both men (OR=0.73, nominal $p=0.02$, empirical $p=0.46$, Bonferroni-adjusted $p=0.48$) and women (OR=0.59, nominal $p=0.03$, empirical $p=0.61$, Bonferroni-adjusted $p=0.72$), though associations were not statistically significant in sex-specific samples. No significant genetic associations were observed for any other candidate genes (i.e. empirical $p>0.05$ and Bonferroni-adjusted $p>0.05$ for all other SNPs).

Because the association with rs1614662 was statistically significant as per Bonferroni adjustment for multiple testing, we further explored the cause of this positive signal by genotyping 4 additional SNPs spanning the GP6 gene, all of which were in moderate linkage disequilibrium with the initial missense SNP and in low-to-moderated linkage disequilibrium with each other ($r^2=0.0$ to 0.46). Figure 1 and Table 3 show results of genetic association of all GP6 SNPs with AMI. In the total sample, an additional SNP (rs11084382) in intron 5 that is in moderate LD with the missense SNP was also associated with AMI (OR=0.74, nominal $p=0.004$). This SNP was also significantly associated with AMI in men (OR =0.77, nominal $p=0.021$) but not women (OR=0.66, nominal $p=0.065$). A third SNP (rs1671207) was associated with AMI in men only (OR=1.33, nominal $p=0.018$).

Because a previous study by Bray and colleagues (14) suggested that AMI risk in post-menopausal women with specific GP6 genotypes differed by their hormone replacement therapy (HRT) status, we tested this hypothesis in our study, but strictly speaking, did not detect a statistically significant interaction (nominal $p=0.06$) between the presence or absence of HRT and genotypes at the rs1613662 missense polymorphism. Nevertheless, we also performed stratified analyses in women who had ever used HRT versus women who had never used HRT. The results in Table 4 indicate that the overall association between variants at the GP6 locus and AMI status among all women appears to be driven by the

association among women having used HRT, and that this association is in a similar direction to that observed in men (Table 3). Women who had never used HRT showed no association with any of the GP6 alleles.

Finally, we performed haplotype analyses to assess whether a specific haplotype influenced risk of AMI. Haplotypes were estimated via an expectation maximization algorithm for the set of 3 SNPs that were individually associated with AMI in the total sample (rs11084382 and rs1613662) or in men (rs1671207). These results indicated that three GP6 haplotypes were significantly associated with AMI, (nominal $p < 0.05$ for each; results not shown). While consistent with the single-SNP results, no single GP6 haplotype was responsible for the relationship with AMI. In other words, haplotypes affirmed the association of the GP6 locus with AMI, but did not identify a specific genetic background that was responsible for altered disease risk.

Discussion

The major finding from this population-based case/control study of AMI survivors was that polymorphisms in GP6 were associated with AMI, whereas genetic variants in other candidate genes were not. Specifically, significant associations of rs1613662 and rs11084382 with AMI were observed, as well as an interaction between HRT with rs1613662 in postmenopausal women. The effect size (OR) of the minor alleles of these SNPs was approximately 0.70, representing a 30% reduction in risk for AMI.

Several published studies have implicated polymorphisms of the GP6 gene in coronary disease (15–17). Contrary to our results indicating that the minor allele of the Ser219Pro missense polymorphism (rs1613662) was protective against AIM, Ollikainen et al. (16) found that sudden death due to AMI was more common among men from Finland (mean age 55 years) who carried the minor allele, although this study comprised 34 cases and 92 controls. A report from Croft et al. on 525 MI patients and 474 controls (mean age = 61 years) in the United Kingdom (15) indicated that after incorporating recognized CHD risk factors, the rs1613662 minor allele was not associated with AMI in men and women overall, however, it was associated with increased AMI in women who were also carriers of a beta-fibrinogen variant. Consistent with our results, Bray et al. (14) observed that women (mean age = 66 years) homozygous for the rs1613662 minor allele may have been at reduced risk of AMI (though this trend was not statistically significant). However, they also reported that the rs1613662 minor allele was associated with increased risk of AMI among women on HRT. We, too, observed an interaction between HRT and GP6 on risk for AMI, however our results indicate that the rs1613662 minor allele was associated with decreased risk of AMI in women who have used HRT. Lastly, consistent with our results, a Japanese study of 376 cases of AMI (including both men and women) and 1080 control participants found that the minor (alanine) allele at the Thr249Ala polymorphism (rs2304167) in GP6, which is in 100% LD with our risk allele at rs11084382 in the HapMap Asian samples (Han Chinese in Beijing, China; Japanese in Tokyo, Japan)(18), conferred a 2.3 fold excess risk of AMI after covariate adjustment (17). These studies, however, have been limited in part by the modest number of participants, the varied definition of the phenotype, the use of clinic patients, the adjustment for various confounders, the lack of community-based study subjects, and/or the use of one or few SNPs within the GP6 locus to test for associations. We speculate that some of the inconsistencies among these studies may be due to survivorship bias. Our study, for example, included as cases only survivors of AMI, whereas Ollikainen et al. looked only at cases of mortality due to AMI. The opposite effects observed in these two studies may be explained by the role of GP6 in survivorship of AMI (i.e. minor allele predictive of AMI death and major allele predictive of AMI survivorship), rather than a direct role in disease pathogenesis. Taken together, these studies consistently show that genetic variation in GP6

is associated with risk of AMI, although it is unclear which variants contribute to this risk and what biological mechanism is responsible.

The present study benefits from several strengths including the large sample of incident cases and community-based controls, the incorporation of several non-genetic risk factors in our models, and the analysis of multiple GP6 SNPs and haplotypes. Haplotype analysis improves power to detect association of a nearby untyped polymorphism compared to single-SNP analysis. Results of our haplotype analyses, which did not point to any specific haplotype(s) as driving the association, suggest that the missense polymorphism (not an untyped variant) may be the major causal variant (although other variants may also contribute to risk).

The results of this investigation are consistent with other studies suggesting that one or more polymorphisms in the GP6 gene influence the risk of AMI and are dependent on the use of HRT in women. There is a sound biological basis for this conclusion as thrombosis/fibrinolysis is involved in the evolution of coronary thrombosis and development of an AMI. Unfortunately, we did not have a measure of an intermediate phenotype to examine whether specific SNPs were functional. However, it has been recently reported (19) that the degree of platelet aggregation was lower in some patients due to variation of the GP6 gene. It is unclear whether this finding is related to the protective effect of the missense polymorphism in our study. A future area of research would be to investigate whether any of these SNPs alter the ability of GP6 to bind to collagen. If polymorphisms in the GP6 locus could be linked with blood concentrations of proteins that are associated with risk of MI, pharmacologic interventions that alter these concentrations could be examined for their role in risk prediction viz-a viz alterations in the intermediate phenotype. The clinical indications for medications that alter platelet function are an active area of interest in this field. Unfortunately, we did not have data to address this question.

Other limitations of this data set include the fact that we examined survivors of AMI and therefore survivorship bias may have been introduced, as described above. Due to the study design, we do not know if the genetic associations present were related to incidence of AMI or case-fatality. This is an outstanding point of uncertainty and will need to be more carefully explored in future studies in order fully elucidate the role of GP6 on AMI.

In summary, we have shown that polymorphic variation at the GP6 locus is associated with risk of AMI in men and women and that this risk is modified by HRT. Our findings suggest that pharmacological approaches to platelet activity and thrombosis should be targeted towards GP6 to aid in the secondary prevention of AMI.

Acknowledgments

We would like to thank the study participants and contributing hospitals as well as the National Heart, Lung, and Blood Institute for funding (R01 HL075389). We would also like to acknowledge the contribution of three anonymous reviewers for their thoughtful consideration of this work.

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Figure 1. Linkage disequilibrium (r^2) between polymorphisms in the GP6 gene
Positions of SNP markers in the GP6 gene are indicated and annotated as missense (i.e. exonic non-synonymous), intronic, or the 3' untranslated region. Linkage disequilibrium among these SNPs is shown in the heat map.

Table 1

Population characteristics of sample

	total sample n=1277		male cases n=504		male controls n=481		female cases n=148		female controls n=144	
	mean	(SD)	mean	(SD)	mean	(SD)	mean	(SD)	mean	(SD)
Demographic										
age (years)	55.5	(8.6)	54.7	(8.9)	54.3	(8.8)	59.0	(6.9)	58.8	(6.8)
gender (% men)	77.1	-	-	-	-	-	-	-	-	-
Anthropometric										
BMI (kg/m ²)	28.7	(4.7)	28.7	(4.2)	28.6	(4.6)	29.1	(5.8)	28.8	(5.6)
obesity (BMI > 30; %)	34.4	-	35.6	-	30.4	-	40.3	-	38.3	-
Smoking										
Never Smoker (%)	32.7	-	37.5	-	22.9	-	51.0	-	29.9	-
Former (%)	51.1	-	44.6	-	63.4	-	32.9	-	51.4	-
Current Smoker (%)	16.2	-	17.9	-	13.7	-	16.1	-	18.8	-
Medical										
Hypertension (%)	39.0	-	41.0	-	31.9	-	52.8	-	43.0	-
Diabetes Mellitus (%)	14.2	-	16.4	-	11.7	-	22.9	-	7.4	-
Hyperlipidemia (%)	66.4	-	57.6	-	67.7	-	71.5	-	85.2	-
Positive Family History										
Heart Disease (%)	31.7	-	37.4	-	24.2	-	36.8	-	33.6	-

Hypertension was defined as $\geq 140/90$ or on antihypertensive medication

Diabetes mellitus was defined by self-report and on antidiabetic medication or fasting glucose >125 mg/dl

Hyperlipidemia was defined by an affirmative answer to "are you currently being treated for high cholesterol?" or total cholesterol >200 mg/dl

Family history of heart disease was defined as a positive history in a first degree relative

Table 2

Genetic association between candidate gene polymorphisms and AMI

gene	marker	MAF	total sample			men			women		
			n	OR	p	n	OR	p	n	OR	p
ABCA1	rs1800976	0.46	1262	1.00	0.96	971	0.95	0.59	291	1.17	0.41
APOE	epsilon 4	0.13	1281	1.14	0.30	987	1.22	0.16	294	0.88	0.67
CD14	rs2569190	0.46	1182	1.06	0.46	909	1.08	0.42	273	1.00	0.99
CHAT	rs10082479	0.11	1242	0.92	0.56	960	0.93	0.65	282	0.97	0.91
chr 15	rs965435	0.28	1212	1.09	0.38	933	1.05	0.64	279	1.22	0.35
chr 22	rs2301523	0.13	1180	0.99	0.97	907	0.97	0.82	273	1.08	0.75
chr 22	rs2331291	0.13	1241	0.96	0.77	956	0.94	0.66	285	1.06	0.82
chr 9	rs2383207	0.45	1236	0.84	0.04	952	0.81	0.03	284	0.91	0.58
chr 9	rs10757278	0.48	1234	0.88	0.13	951	0.83	0.06	283	1.00	0.99
CRP	rs1130864	0.33	1270	0.94	0.49	981	0.99	0.94	289	0.75	0.16
CRP	rs1800947	0.07	1268	0.95	0.76	977	1.02	0.92	291	0.72	0.35
CRP	rs2794521	0.26	1266	1.10	0.30	977	0.98	0.85	289	1.64	0.01
E-selectin	rs5355	0.04	1277	1.01	0.96	984	1.04	0.87	293	0.87	0.75
E-selectin	rs5368	0.10	1248	0.85	0.24	959	0.89	0.47	289	0.73	0.32
FL3A1	rs5985	0.26	1214	0.92	0.41	934	0.91	0.36	280	0.97	0.88
F3	rs1361600	0.49	1259	0.96	0.58	967	0.90	0.29	292	1.15	0.42
F3	rs3761955	0.48	1263	0.94	0.49	973	0.91	0.32	290	1.08	0.67
GPIBA	rs2243093	0.12	1257	1.00	0.99	967	1.10	0.51	290	0.68	0.19
GP6	rs1613662	0.16	1268	0.70	0.002	976	0.73	0.02	292	0.59	0.03
IL10	rs1800896	0.46	1270	1.01	0.93	978	0.98	0.81	292	1.14	0.44
IL18	rs187238	0.28	1271	1.10	0.29	980	1.09	0.40	291	1.09	0.65
IL1A	rs1800587	0.30	1258	1.02	0.83	974	1.01	0.92	284	1.15	0.49
IL1B	rs16944	0.33	1268	0.92	0.35	977	0.96	0.68	291	0.81	0.27
IL8	rs4073	0.45	1225	1.01	0.90	947	1.04	0.69	278	0.90	0.57
PON1	rs662	0.27	1271	1.18	0.08	980	1.15	0.18	291	1.32	0.17
P-selectin	rs6136	0.10	1270	0.75	0.04	979	0.63	0.01	291	1.21	0.50
TBXAS1	rs764745	0.44	1261	1.05	0.60	971	1.11	0.29	290	0.89	0.51
THBS4	rs1866389	0.22	1263	1.05	0.62	972	1.04	0.73	291	1.16	0.51

gene	marker	MAF	total sample			men			women		
			n	OR	p	n	OR	p	n	OR	p
THBS4	rs2228262	0.10	1264	1.07	0.59	975	1.07	0.64	289	1.11	0.70
TNF	rs1800629	0.16	1263	0.93	0.54	973	0.94	0.65	290	0.92	0.73
TSHB2	rs11758161	0.17	1257	0.95	0.67	967	0.97	0.84	290	0.97	0.89

MAF=minor allele frequency

OR=odds ratio with respect to the major allele

p=nominal (unadjusted) p-value; see text for Bonferroni-adjusted and empirical p-values for select SNPs.

All tests for genetic association are adjusted for covariates indicated in Table 1

Table 3

Genetic association of GP6 polymorphisms with AMI

total sample			men				women			
gene	marker	Minor allele	MAF Cases n=652	Controls n=625	OR	p	MAF Cases n=504	Controls n=481	OR	p
GP6	rs1654410	C	0.506	0.497	0.983	0.841	0.521	0.490	1.062	0.534
GP6	rs11084382	A	0.199	0.252	0.745	0.004	0.191	0.239	0.766	0.021
GP6	rs11669150	C	0.244	0.219	1.181	0.104	0.24	0.222	1.154	0.221
GP6	rs1613662	C	0.133	0.181	0.702	0.002	0.130	0.174	0.727	0.016
GP6	rs1671207	C	0.227	0.196	1.201	0.080	0.236	0.185	1.329	0.018

MAF=minor allele frequency

OR=odds ratio with respect to the major allele

p=nominal (unadjusted) p-value

All tests for genetic association are adjusted for covariates indicated in Table 1

Table 4

Genetic association of GP6 polymorphisms with AMI in women with and without HRT

		Women with HRT (n=152)				Women without HRT (n=140)				
gene	marker	Minor allele	MAF Cases n=81	Controls n=71	OR	p	MAF Cases n=67	Controls n=73	OR	p
GP6	rs1654410	C	0.399	0.541	0.570	0.042	0.515	0.500	0.989	0.968
GP6	rs11084382	A	0.257	0.333	0.511	0.038	0.196	0.226	1.060	0.869
GP6	rs11669150	C	0.295	0.175	2.255	0.011	0.225	0.258	0.809	0.507
GP6	rs1613662	C	0.143	0.228	0.361	0.005	0.144	0.164	1.096	0.810
GP6	rs1671207	C	0.200	0.237	1.083	0.803	0.194	0.226	0.931	0.831

MAF=minor allele frequency

OR=odds ratio with respect to the major allele

p=nominal (unadjusted) p-value

All tests for genetic association are adjusted for covariates indicated in Table 1