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Genetic polymorphisms in platelet-related proteins and coronary artery disease: investigation of candidate genes, including *N*-acetylgalactosaminyltransferase 4 (GALNT4) and sulphotransferase 1A1/2 (SULT1A1/2)

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Abstract *Background* Both platelet function and heart disease show strong genetic components, many of which remain to be elucidated. *Materials and methods* The roles of candidate polymorphisms in ten platelet-associated genes were compared between 1,237 Acute Coronary Syndrome (ACS) cases (with myocardial infarction and unstable angina) and 386 controls, from an Irish Caucasian population. Additionally, 361 stable angina patients were investigated. Two genes of interest were followed up in a separate Irish study of 1,484 individuals (577 with IHD and 907 unaffected). *Results* The *GALNT4* (*N*-acetyl galactosaminyl transferase 4) 506I allele was significantly underrepresented in ACS (OR = 0.66, CI = 0.52–0.84; $P = 0.001$; $P = 0.01$ after correction for multiple testing), while the *SULT1A1* (Sulphotransferase 1A1) 213H allele was associated with risk of ACS (OR = 1.37, CI = 1.08–1.74; $P = 0.01$; $P = 0.1$ after correction for multiple testing). Subsequent genotyping of further SNPs in *GALNT4* in the family-based (IHD) group revealed that the 506I allele showed the same trend towards protecting against ACS but the haplotypic test over the four commonest haplotypes was not significant ($P = 0.55$). In contrast, the *SULT1A1/SULT1A2* gene complex showed suggestive haplotypic association in the family-based study ($P = 0.07$), with the greatest increase in risk conferred by the *SULT1A2* 235T allele ($P = 0.025$). *Conclusion* We have identified two risk genes for cardiovascular disease, one of whose (GALNT4) effects may be on either platelet or endothelial function through modifications of PSGL1 or other important glycosylated proteins. The role of sulphotransferases (SULT1A1/2) in cardiovascular disease requires further exploration. Further validation of cardiovascular risks conferred by both genes in other populations (including gene copy number variation) is warranted.

Keywords Platelets · SULT1A1 · GALNT4 · Genotyping · Cardiovascular genetics

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Introduction

There is a significant genetic component to both cardiovascular events and to variation in platelet function, which is independent of other known risk factors [1]. Some polymorphisms in well known platelet proteins have been associated with coronary artery disease, acute coronary syndromes and myocardial infarction (MI) [2–4], and

differences in the response of individuals to antiplatelet therapies have been linked to genetic variation in drug targets and other related platelet proteins [5]. Currently, the role of many proteins found within the platelet proteome is poorly understood and so it would be of great interest to discover genetic associations between cardiovascular disease and such platelet proteins. This encourages the investigation of all types of platelet proteins from the well-characterized receptor glycoproteins, such as integrins and their ligands, to the smaller, less well-characterized, intracellular proteins.

Here, we identified ten genetic variants in platelet expressed proteins (or platelet protein ligands) as candidates to investigate their association with cardiovascular disease risk. These genes were ALAD (delta-aminolevulinate dehydratase), two collagen genes (COL1A1 and COL3A1), *N*-acetyl galactosaminyl transferase 4 (GALNT4) which modifies P-selectin ligand, CD109 (platelet alloantigen Gov), platelet glycoprotein VI (GPVI), glutathione S-transferase omega 1(GSTO1), Rab geranylgeranyl transferase subunit α (RABGGTA), Sulphotransferase 1A1 (SULT1A1), and thrombospondin 1 (THBS1). The likely functional impact of their polymorphisms, in terms of modifying protein structure, function or expression either in vitro or in vivo, are summarised also.

The ten platelet-related variants were investigated to determine whether they confer genetic susceptibility to CAD in an Irish case–control study. Their association with platelet function assays [6] in a cardiovascular disease population was also investigated. The two genes which were found to be associated with disease (GALNT4 and SULT1A1) in the case–control study were then tested (along with other SNPs from the same genes) for confirmation in a family based study of ischaemic heart disease (IHD), and this provided further evidence for association of the SULT1A1/2 gene region with cardiovascular disease.

Materials and methods

The information and genetic samples provided by all the individuals in this study were obtained with written informed consent in accordance with the approval of the institutional Ethics Committees of the hospitals and/or universities involved.

Case control study

This study is outlined elsewhere [7]. ACS:1,237 subjects with a history of acute coronary syndromes (ACS), categorised as either unstable angina (UA) and myocardial infarction (MI). Unstable angina ($n = 336$) was defined as chest pain typical of angina occurring at rest with duration of at least 20 min and requiring hospitalisation in a patient with known CAD based on coronary angiogram or a positive stress test. MI ($n = 901$) was defined as the occurrence of chest pain for at least 20 min duration along with previous or current electrocardiogram and/or serum enzyme changes diagnostic of MI. Age, gender, smoking history, history of medication for hypertension, hypercholesterolemia or diabetes were also recorded (Table 1).

Stable angina comparison group

We also genotyped a stable angina group for comparison purposes. Stable angina (SA) ($n = 361$) was diagnosed as chest pain occurring with exercise, typical of angina in a patient with known coronary artery disease (CAD) based on coronary angiogram or a positive treadmill test.

All patients in the ACS and stable angina groups were consecutive/selected.

Controls

About 386 individuals were employees of an Irish financial institution (Table 1).

Family-based ischaemic heart disease (IHD) study

Recruitment of the participants ($n = 1{,}484$) took place between August 1999 and October 2004. The inclusion criteria are described in detail in a previous publication [8]. Briefly, individuals were Caucasian with all four grandparents born in Ireland. Each family was required to have at least one family member affected with proven premature IHD (disease onset $\leq$55 years for males and $\leq$60 years for females) and at least one unaffected sibling and/or both parents surviving. Proven IHD was defined by one or more of the following: previous myocardial infarction (MI), previous unstable angina (typical chest pain with dynamic ECG changes or minor elevation of cardiac markers) or stable angina with angiographic evidence of obstructive coronary disease (>70% stenosis). Unaffected siblings were required to be 3 years older than the affected sibling at age of diagnosis of IHD and have no evidence of previous IHD using the "Rose chest pain on effort and possible infarction questionnaire" [9] and standard 12 lead electrocardiogram independently coded using the "Minnesota code" [10].

Table 1 Comparison of ACS case and control populations

	Acute coronary syndrome study population		Control population	
Gender				
Male/female	1,232 (77.1%)	366 (22.9%)	225 (58.3%)	161 (41.7%)
Age				
<55 years (male) or <60 years (female)/≥55 years (male) or ≥60 years (female)	697 (43.9%)	890 (56.1%)	329 (85.2%)	57 (14.8%)
Smoking status				
Current or previous/Never	1,205 (76.2%)	376 (23.8%)	116 (30.4%)	265 (69.6%)
Hypertension requiring medication				
Yes/No	658 (41.3%)	935 (58.7%)	–	–
Hypercholesterolemia requiring medication				
Yes/No	935 (58.7%)	657 (41.3%)	–	–
Diabetes mellitus requiring medication				
Yes/No	148 (9.3%)	1,444 (90.7%)	–	–

Aspirin study

This small population ($n = 144$) of individuals was aged 21 years and over with a documented history of cardiovascular disease but who were currently stable. They were all taking aspirin (75–300 mg) once daily for at least 2 weeks and were on no other non-steroidal anti-inflammatory drugs, cyclooxygenase inhibitors, anti-platelet therapies or anticoagulant medications. At least 2 weeks compliance with aspirin was emphasised and was recorded [6, 11].

Choice of single nucleotide polymorphisms (SNPs) investigated

References providing evidence that each variant studied confers a variation in platelet-related protein function, sequence or expression are shown in Table 2.

Analysis of the ten platelet-related variants in the ACS-control population yielded significant associations involving the SULT1A1 R213H and GALNT4 V506I polymorphisms. Therefore, in an effort to establish whether alleles/haplotypes at the GALNT4 or SULT1A loci were associated with IHD, we selected additional polymorphisms in the region on the SULT1A1 and GALNT4 genes, flanking these two variants. Along with the original SULT1A1 R213H variant, four SNPs were chosen in the SULT1A1 and SULT1A2 genes, which using data taken from HAPMAP as well as previously published data [12], are in linkage disequilibrium with the SULT1A1 R213H variant. They were SULT1A2 L19P, SULT1A2 T235N, SULT1A2 *INT2*G/A and SULT1A2 *INT5*G/A. Similarly in the GALNT4 gene two polymorphisms, GALNT4 T270I and GALNT4-8755A/G, which with the use of HAPMAP were shown to be in linkage disequilibrium with the original GALNT4 V506I variant were also genotyped. Additional SULT1A and GALNT4 polymorphisms from dbSNP (database of single nucleotide polymorphisms [13]) were investigated but were found not to be polymorphic in the Irish population under study here.

Genotyping methodology

All polymorphisms investigated were SNPs. Genotyping was performed by KBiosciences Ltd, UK in 384-well microplates using a fluorescence resonance energy transfer (FRET)-based, competitive allele-specific PCR (CASP) genotyping method. Three nanogram of genomic DNA provided the template for each genotyping assay. Amplification was initiated using allele-specific primers and a common downstream primer. The allele-specific primers were tailed with unique sequences that created corresponding complementary sequences in the two amplicons. In the second round of amplification, quenched Universal Amplifluor^{TM} primers in a hairpin formation were used. These primers contained 3′ tails that specifically bind to the unique tailed sequences in the amplicons, and continue amplification.

Platelet aggregation studies

Platelet aggregation studies were performed on blood platelet samples collected from 144 stable angina patients

Table 2 Risk of ACS versus controls conferred by alleles from platelet related genes

Gene and SNP reference	Genotype[a]	Control (%)	ACS (%)	MI[b] (%)	SA (%)	Functional effect of variant	Odds ratio (95% confidence interval)	*P* value
ALAD K59N	GG	84.8	85.2	85.3	87.4	59N allele disrupts catalytic activity of the enzyme [17, 18]	0.96 (0.70–1.32)	0.81
(delta-Aminolevulinate Dehydratase)	**GC**	15.0	14.5	14.4	12.0			
rs1800435	**CC**	0.2	0.3	0.3	0.6			
CD109 Y703S	CC	28.2	29.3	29.8	28.5	Encodes alternate alleles of the Gov a/b antigen system [19]	0.96 (0.74–1.23)	0.73
(CD109Antigen/Gov platelet alloantigen)	**CA**	53.3	48.5	48.2	51.0			
rs10455097	**AA**	18.5	22.2	22.0	20.5			
COL1A1 INT1 + 1240G/T	GG	64.6	65.7	66.8	62.3	T allele binds Sp1 with a higher affinity, leads to 3-times higher rate of transcription [20, 21]	0.95 (0.74–1.21)	0.66
(Collagen Type 1, Alpha 1)	**GT**	33.0	31.0	30.2	33.5			
rs1800012	**TT**	2.4	3.3	3.0	4.2			
COL3A1 A531T	GG	60.3	58.4	58.6	58.9	May alter collagen type III mediated platelet signalling at sites of vascular injury or lesion [22]	1.07 (0.85–1.35)	0.57
(Collagen Type 3, Alpha 1)	**GA**	35.0	36.9	36.2	34.6			
rs1800255	**AA**	4.7	4.7	5.2	6.5			
GALNT4 V506I	GG	33.8	43.8	44.1	40.4	506I allele is expressed at lower levels and shows reduced PSGL-1 substrate specificity [23]	0.66 (0.52–0.84)	**0.001**
(UDP-N-acetylgalactosamine polypeptide-N-acetyl	**GA**	53.5	43.5	43.7	46.6			
galactoseaminyltransferase 4)	**AA**	12.7	12.7	12.2	13.0			
rs2230283								
GPVI S219P	TT	71.9	72.1	72.2	70.1	219P allele associated with reduced receptor density, platelet adhesion and thrombus formation [24, 25]	0.98 (0.76–1.26)	0.85
(Platelet Glycoprotein VI)	**TC**	26.3	26.0	25.6	27.4			
rs1613662	**CC**	1.8	1.9	2.2	2.5			
GSTO1 A140D	CC	54.2	49.8	49.3	52.9	140D allele exhibits only 75% of wild type activity [26, 27]	1.18 (0.94–1.49)	0.16
(Glutathione S-transferase, Omega-1)	**CA**	39.7	41.3	41.5	38.2			
rs4925	**AA**	6.1	8.9	9.2	8.9			
RABGGTA T420A	AA	42.6	39.8	40.9	43.8	Gene involved in correct platelet synthesis, effect of variant unknown [28–30]	1.13 (0.90–1.43)	0.30
(Rab geranylgeranyl transferase subunit alpha)	**AG**	47.3	46.9	45.6	45.5			
rs729421	**GG**	10.1	13.3	13.5	10.7			
SULT1A1 R213H	GG	50.3	42.2	43.0	43.9	213H exhibits lower thermostability and 7.7-fold lower activity [31, 32]	1.37 (1.08–1.74)	**0.01**
(Sulfotransferase family, cytosolic, 1A, phenol	**GA**	45.9	46.4	45.8	44.5			
preferring, member 1)	**AA**	3.8	11.4	11.2	11.6			
rs9282861								
THBS1 N700S	AA	76.9	76.4	75.2	76.7	700S is thermolabile and protein levels are reduced in plasma [33, 34]	1.04 (0.79–1.37)	0.78
(Thrombospondin 1)	**AG**	21.8	21.7	22.7	21.9			
rs2228262	**GG**	1.3	1.9	2.1	1.4			

[a] Bold indicates two genotypes merged into one group for calculation of OR

[b] Note that MI (myocardial infarction) is a subset of the ACS group

and prepared as described in Maree et al. [6, 11]. Levels of thromboxane B_2 (TXB_2) in serum and of arachidonic acid induced platelet aggregation were recorded and used to measure drug resistance i.e. the level of platelet activation that persists in the presence of aspirin. Platelet aggregation assays were performed to determine whether levels of thromboxane B_2 (TXB_2) production in serum and arachidonic acid-mediated platelet aggregation were associated with carriership of the platelet-associated risk alleles under study. Arachidonic acid induced platelet aggregation of 20% or more has previously been shown to be clinically important [14] and thus a 20% or greater increase over baseline in aggregation to arachidonic acid was classified as high aggregation. High TXB_2 was defined as serum levels of TXB_2 of 2.2 ng/ml or greater, as this indicates evidence of sub-optimal COX-1 inhibition [6, 11].

Statistical analysis

Genotypes were grouped for analysis, primarily to avoid testing rare genotypic classes which have limited power for the detection of effects. In each case, the rarer homozygote was pooled with the common heterozygote. For each locus, the genotypes were combined into two groups (as shown in Table 2). The choice of dominance/recessivity for each allele was based on the similarity of biological effect observed in previous studies of platelet function, or secondarily on the basis of pooling the two rarer genotypes. All logistic regression and related analyses were performed using the STATA (Version 8: StataCorp 2003) [15].

In the IHD population, haplotypic associations and tests of linkage disequilibrium were carried out using TRANSMIT (version 2.5.4). This program measured association between the SULT and GALNT4 polymorphisms/haplotypes and disease, by examining the observed versus estimated transmission of alleles from parents to affected offspring. The presence of phase uncertainty in haplotypes meant that numbers of observed transmissions had to be estimated. The primary endpoint for these validation studies was whether the common haplotypes (with rarer haplotypes pooled as one class) showed a significant bias in transmission to cases. This study design avoids the problem of population stratification found in some case–control studies. Such stratification was shown to be relatively low in the ACS case–control group presented here [11].

Logistic regression analysis was utilized to compare allele carriership for each variant among high aggregation (20% increased aggregation over baseline in response to arachidonic acid) versus low aggregation patients, and among those patients with high TXB_2 levels (>2.2 ng/ml) versus low TXB_2 levels.

Results

Primary analysis—study of control and coronary artery disease populations

Error rates for each of the ten genotyping assays were calculated based on duplicate genotyping of 87 samples randomly chosen from the entire population. This revealed that only one of the ten assays, that of SULT1A1, had a discordance rate of 2.3%. This gave a combined discordance rate over all ten platelet-associated SNPs of 0.23%.

The primary hypothesis was to test whether each of the ten variants showed a difference in frequency between ACS and controls, with Bonferroni correction for multiple testing. Analysis of the impact of the ten platelet-associated variants on disease outcome is summarized in Table 2.

Logistic regression analysis was carried out for each of the ten platelet-associated genes for the ACS group versus controls. Carriership of each individual SNP was analysed for risk of ACS compared to controls. Of the ten SNPs the GALNT4 506I carriers had a significantly reduced risk of ACS compared to controls (Odds ratio [OR] = 0.66, 95% confidence interval [CI] = 0.52–0.84, $P = 0.001$). Similarly carriers of the GALNT4 506I allele had a lower risk of MI (OR = 0.65, CI = 0.50–0.84, $P = 0.001$) and overall CAD (ACS + SA) (OR = 0.68, CI = 0.53–0.86, $P = 0.001$) compared to controls. Carriers of the SULT1A1 213H allele had an increased risk of ACS (OR = 1.37, CI = 1.08–1.74, $P = 0.01$), MI (OR = 1.33, CI = 1.03–1.70, $P = 0.027$) and overall CAD (OR = 1.36, CI = 1.08–1.72, $P = 0.009$). Within the CAD population itself, no differences were found in any of the ten platelet-related variants between the ACS and SA groups (Table 2).

Following Bonferroni correction for multiple testing of the primary hypothesis (ACS versus controls), statistical significance was maintained for the GALNT4 506I allele ($P_c = 0.01$) but was lost for the SULT1A1 213H allele ($P_c = 0.1$).

Platelet function assays

Our objective was to determine if the GALNT4 506I allele protected against platelet aggregation, and whether the SULT1A1 213H allele increased risk of platelet aggregation, since they were selected as candidate risk factors on the basis of their presence in platelets, and respectively protect against, and increase risk of, heart disease. The GALNT4 506I allele had a non-significant protective effect against elevated arachidonic acid induced aggregation (OR = 0.43, CI = 0.17–1.08). There is no association with serum TXB_2 (OR = 1.01), indicating that the genetic

Table 3 Platelet function assays and association with ten platelet-associated variants in stable angina patients ($n = 144$)

Gene	Carrier allele	Serum TXB2			Arachidonic acid aggregation		
		OR	95% CI	*P* value	OR	95% CI	*P* value
ALAD K59N	C	1.43	0.43–4.74	0.56	–	–	0.02[a]
CD109 Y703S	A	1.55	0.53–4.47	0.42	1.16	0.48 – 2.81	0.74
COL1A1 *INT1* + 1240G/T	T	2.42	1.00–5.88	0.05	2.72	1.03 – 7.18	0.04
COL3A1 A531T	A	0.85	0.35–2.10	0.73	1.48	0.64 – 3.45	0.36
GALNT4 V506I	A	1.01	0.41–2.49	0.99	0.43	0.17 – 1.08	0.07
GPVI S219P	C	0.96	0.38–2.44	0.94	1.26	0.53 – 3.01	0.60
GSTO1 A140D	A	1.60	0.66–3.87	0.30	1.10	0.49 – 2.47	0.81
RABGGTA T420A	G	0.65	0.27–1.57	0.34	0.78	0.34 – 1.80	0.57
SULT1A1 R213H	A	1.52	0.60–3.83	0.37	1.68	0.74 – 3.82	0.22
THBS1 N700S	A	0.98	0.35–2.71	0.97	0.53	0.23 – 1.27	0.16

Logistic regression of the ten platelet-associated alleles with high versus low serum TXB2 levels and high versus low platelet aggregation in response to arachidonic acid

[a] Odds ratio and confidence intervals could not be calculated for the effect of the ALAD-59N allele on arachidonic acid-mediated platelet aggregation as all carriers of this low frequency allele exhibited increased levels of platelet aggregation; *P*-value from Fisher's exact test

variant is not influencing the extent of aspirin resistance. The SULT1A1 213H allele, which was suggested above to cause an increase in risk of ACS, was weakly but not significantly associated with higher arachidonic acid induced platelet aggregation (Table 3). We noted an apparently stronger association of the COL1A1 *INT1*T allele with both arachidonic acid aggregation (Table 3), and with serum thromboxane levels, suggesting the hypothesis that collagen genetic variation could influence platelet function in these assays.

Family based IHD study

The primary endpoint pre-assigned was the test of departure from expectation of major haplotypes across the SNPs genotyped in each gene region. The overall haplotypic association was not significant for GALNT4 ($P = 0.54$, Table 4) and only suggestive for the SULT1A1/SULT1A2 gene complex ($P = 0.07$). It was however notable that one of the SULT1A2 SNPs demonstrated a relatively strong association (235T, 455 observed transmissions to the affected compared to 433.6 expected, $P = 0.025$). The rationale for genotyping SNPs from SULT1A2 was that the pattern of linkage disequilibrium [16] around SULT1A1 extended to the neighbouring SULT1A2 gene. It is difficult to infer exactly which allele is more likely to be causative in this context. Indeed, Table 4 indicates that the main difference relates to an excess of the 213H/235N/*INT5*G haplotype and a deficit of the 213R/235T/*INT5*G haplotype, whereas the commonest haplotype 213R/235T/*INT5*A shows no difference.

Discussion

We investigated genetic variants in platelet-associated proteins, to determine whether they influenced the risk of developing ACS. This revealed that two gene regions in particular, *GALNT4* and *SULT1A1/2*, appeared to influence the risk of ACS compared to controls. SULT1A1 was selected in this study on the basis that it is present in platelets, and demonstrates marked variation in functional activity [31], while GALNT4 was selected on the basis of its role in modifying glycoproteins, which play a critical role in both platelet and endothelial cells. While the original hypothesis regarding genotypic effects came from postulated effects in platelets, it is possible that the effects are mediated through effects on platelet function, or alternatively through some entirely different mechanism of action. To investigate this, we explored allele frequencies in a small study which had information on platelet function, the Aspirin study. In this group some suggestive evidence was found for the association of GALNT4 allele carriership with elevated platelet aggregation in response to arachidonic acid. Although replication in a larger population with similar measurements of platelet function would be warranted. Having established risks in the Irish ACS population we then investigated these in the family based study which helps avoid concerns about potential biases in choice of controls. Since the two candidate genes of interest for immediate follow-up were associated with both stable and unstable disease, it was appropriate to consider both together in the follow up study. For this reason, in the analysis of the family based study, the outcomes of acute coronary syndromes (MI and unstable angina) and stable

Table 4 GALNT4 and SULT1A1/1A2 analysis of IHD family-based genotype data using TRANSMIT

(a) Single marker analysis

SNP	SNP rs number	Allele	Observed (%)	Expected (%)	χ^2(1 df)	*P*-value
GALNT4 V506I	rs2230283	G	754 (66.37)	743.89 (65.48)	1.24	0.27
		A	382 (33.63)	392.11 (34.51)	1.24	0.27
GALNT4T270I	rs2230281	C	842 (71.53)	834.85 (72.47)	1.53	0.22
		T	328 (28.47)	317.15 (27.53)	1.53	0.22
GALNT4–8755 A/G	rs1565679	A	855 (74.61)	867.75 (75.72)	2.33	0.13
		G	291 (25.39)	278.25 (24.28)	2.33	0.13
SULT1A1 R213H	rs9282861	G	727 (65.61)	741.16 (66.89)	2.47	0.12
		A	381 (34.39)	366.84 (33.11)	2.47	0.12
SULT1A2 T235N	rs1059491	C	455 (39.29)	433.64 (37.45)	5.04	0.025
		A	703 (60.71)	724.36 (62.55)	5.04	0.025
SULT1A2-*INT5* G/A	rs3743963	G	635 (55.03)	627.34 (54.36)	0.63	0.43
		A	519 (44.74)	526.66 (45.64)	0.63	0.43

(b) Multi marker haplotype analysis

Haplotype	Observed (%)	Expected (%)	Haplotype *p*-value
GALNT4			
506I/270T/−8755-A	441.7 (37.8)	445.0 (38.1)	0.73
506V/270T/−8755-A	394.0 (33.7)	401.3 (34.4)	0.43
506I/270I/−8755-G	294.9 (25.2)	282.9 (24.2)	0.16
506I/270I/−8755-A	36.4 (3.1)	36.9 (3.2)	0.89
506I/270T/−8755-G	1.1 (0.1)	1.9 (0.2)	–
	Composite test in common haplotypes (3 df) $P = 0.54$		
SULT1A1/SULT1A2			
213R/235T/*INT5*-A	523.6 (45.1)	527.9 (45.4)	0.97
213H/235N/*INT5*-G	412.2 (35.5)	392.6 (33.8)	0.039
213R/235T/*INT5*-G	177.0 (15.2)	193.5 (16.7)	0.026
213R/235N/*INT5*-G	43.9 (3.7)	42.2 (3.6)	0.65
213H/235T/*INT5*-G	2.1 (0.2)	2.6 (0.2)	–
213H/235T/*INT5*-A	2.1 (0.2)	2.6 (0.2)	–
213R/235N/*INT5*-A	1.0 (0.1)	0.7 (0.1)	–
	Composite test in common haplotypes (3 df) $P = 0.07$		

disease (stable angina) were considered together in statistical testing of genotypic effects.

A second stage genotyping in a separate IHD population suggested that the SULT1A1/2 locus also shows some association. Since the SULT1A1/2 genetic association pattern is not attributable to a single SNP, this suggests that the important variation is associated with certain haplotypes, and is likely to be encoded by an unmeasured genetic variant. One candidate source of variation is the gene copy number variation seen in this gene complex [35]. However, one important caveat needs to be sounded in relation to the interpretation of the SULT1A1/2 data presented here. We originally typed another two SNPs, SULT1A2 L19P (rs10797300) and SULT1A1 *INT*2G/A (rs12445705), and found notable transmission errors in these SNPs. For these reasons, we excluded these SNPs from this analysis. While these errors could arise from assay errors, it is also possible that gene copy number polymorphism can distort apparent transmission patterns of SNPs. Other workers have noted a potential gene copy number polymorphism at SULT1A1/2 locus [35]. Therefore, it is possible that the genotypic picture is not complete, since SNP typing may not capture important variation in gene copy number. We surveyed the SNP allele frequencies across various Caucasian studies to determine if particular assays or populations may be more sensitive to distortions that could reflect gene copy number differences. In general, the studies are fairly similar, with greatest frequency differences seen in the smaller studies. This suggests that copy number variation, if it exists to any

great extent, does not appear to have markedly distorted allele frequencies in general. SNP associations may reflect haplotypic associations, or may be associated with gene copy number differences.

While we found a strong association of GALNT4 in the ACS population, this finding was not significant in the follow-up IHD family based association study. This may reflect small population size, population clinical heterogeneity, or a study design flaw such as poor matching of cases and controls. The control group chosen was primarily selected on the basis of availability and ethical approval for use in studies of cardiovascular disease. Clearly, a larger population-based (rather than professional based) control group would characterize the risks identified more clearly. The existing dataset has $\sim$80% power to detect an association between ACS and the SULT1A1 R213H SNP with an odds ratio of 1.3: if a more balanced design had been chosen (800 cases and 800 controls) the power rises to $\sim$90%. However, our study power drops to 45% once a stricter P-value is imposed ($P = 0.005$, allowing for ten separate tests). Low power in genetic studies clearly has the potential to identify spurious false positive findings and therefore the findings presented here need to be interpreted primarily as initial hypothesis generating observations, and may well be of greatest interest to researchers already following up other strands of evidence in relation to the role of these candidate proteins in cardiovascular disease. While there is relatively little population genetic substructure among Caucasians of Irish origin, there would be some potential for slight improvement in study design in terms of ensuring control individuals are matched more closely for grandparental county of origin [36].

GALNT4 has been shown to preferentially *O*-glycosylate the threonine residues at position 44 and 57 of the P-selectin glycoprotein ligand (PSGL-1) [23]. The function of PSGL-1 is as a counter-receptor for P-selectin, which is stored in the Weibel–Palade granules of endothelial cells and the α granules in platelets [37]. PSGL-1 interacts with P-selectin and is critical in the rolling and tethering of platelets and leukocytes on activated endothelial surfaces and is thus important in inflammatory and thrombotic reactions [38]. In a study by Liu et al. [39], the Thr^{57} residue of PSGL-1 was shown to be an *O*-glycan carrier site required for P-selectin binding. Therefore, it is possible that *O*-glycosylation of PSGL-1 by GalNAc-T4 may be important for many P-selectin mediated cell–cell interactions. Bennett et al. [23] reported that expression of the 506I allozyme in insect cell lines was lower than that observed for the wild-type protein and although kinetic studies revealed similar activities for both variants in vitro, a comparison of enzyme specificities was not performed due to low yields of the 506I allozyme. Since the V506I polymorphism occurs in the lectin-like domain of GalNAc-T4, which has been shown to confer substrate specificity [40, 41], the 506I variant may indeed exhibit altered preference for its substrate PSGL-1. Alternatively it may be in linkage disequilibrium with a regulatory polymorphism, which reduces its expression and this could lead to diminished binding of PSGL-1 to P-selectin.

How might the SULT1A1/2 gene complex modulate cardiovascular risk? SULT1A1 catalyses the transfer of a sulpho moiety to hydroxyl containing substrates [42], including steroids, catecholamines, peptides, and xenobiotics [42–44]. However sulphation can also lead to the generation of unstable electrophilic conjugates which are mutagenic and readily form DNA and RNA adducts [45, 46]. Tissue sulphotransferase enzyme expression and activity varies widely in the human population and there is good evidence to suggest that this variation is at least in part genetically determined. Variation in enzyme activity, thermostability and protein level has been shown to be associated with the R213H polymorphism in *SULT1A1* [31, 32, 44, 47], and activity is higher in females than males ($P = 0.001$) [47]. Individuals homozygous for the 213H genotype express a more thermolabile protein with an enzyme activity 7.7-fold lower than that observed for 213R homozygotes [32]. Since possession of the SULT1A1-213H allele appears to associate with risk of ACS and MI in this study, one could postulate that sulphation of platelet compounds, may lead to increased platelet activation. Although the small population of cardiovascular disease patients with platelet function activity showed a non-significant association (Table 3), it would be of interest to investigate the role of SULT1A1/2 polymorphisms in a non-disease population with no anti-platelet therapy, where such genotypic effects may be very different.

In conclusion, we present evidence that GALNT4 and the SULT1A1/2 genes may play a role in the progression of heart disease. Further studies are warranted, in particular looking at their impact on platelet function in aspirin free non-diseased populations, and investigating the roles of gene copy number polymorphism in SULT1A1/2 [35].

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