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# Association between the risk of coronary artery disease in South Asians and a deletion polymorphism in glutathione *S*-transferase M1

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South Asians living in Western societies show a greater risk of coronary artery disease (CAD) than the indigenous Caucasian population, probably related to the change to a Westernised lifestyle and an associated genetic susceptibility. Modulation of DNA damage and mutation caused by polymorphisms in detoxification enzymes, including the glutathione *S*-transferases (GSTs), is a well-established risk factor for tobacco-related carcinogenesis, and a similar change in cellular damage may be involved in the risk of vascular disease associated with tobacco smoking. In this study we examined whether polymorphisms in GST genes influence the risk of CAD in a case-control group of South Asians, following our recent observation of such an association in Caucasians from the same region of the UK. Blood was obtained from 170 patients of South Asian origin admitted for angiographic investigation of chest pain and from 203 controls. Patients were subdivided into those with and without previous acute myocardial infarction (AMI), and DNA was analysed for deletions in the *GSTM1* and *GSTT1* genes. An association was found between the prevalence of the *GSTM1* null genotype and the risk of developing CAD in this study population. The frequency of the null genotype was 52.7% in healthy controls and 41.2% in patients (odds ratio [OR] 0.63, 95% confidence interval [95% CI] 0.42–0.95, $p = 0.029$). The effect was similar in subjects with or without a prior history of AMI. The association was also independent of smoking history, with both non-smokers and smokers showing a similar pattern of genotype distribution, the frequency of the null genotype being 51.2% in controls versus 37.0% in patients in 'never' smokers (OR 0.56, 95% CI 0.33–0.94, $p = 0.037$) and 60.0% in controls versus 46.2% in patients in 'ever' smokers (OR 0.57, 95% CI 0.25–1.28, $p = 0.223$). The association remained after adjusting for age, sex, body mass index and the presence or absence of stenosis. No significant associations were observed between the *GSTT1* genotype and cardiovascular disease ($\chi^2$ test, $p > 0.1$). The results of this study indicate that the *GSTM1* null genotype is protective against both CAD and AMI. However, further study is required in order to elucidate the, as yet unexplained, mechanisms underlying this association.

*Keywords*: glutathione *S*-transferase, genetic polymorphisms, coronary artery disease, South Asians.

## Introduction

Migrant populations moving from South Asia to Europe and North America have higher rates of coronary artery disease (CAD) than the indigenous Caucasian

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populations (Cappuccio 1997). The reasons for this effect remain largely unclear but appear to be related to the change to a Westernised lifestyle and the associated development of central obesity, insulin resistance and hyperlipidaemia (Anand *et al*. 2000). In addition, the migrant populations show abnormalities in their profile of risk factors such as high circulating homocysteine, lipoprotein (a), fibrinogen and plasminogen activator inhibitor-1, and genetic factors are also likely to be important (Anand *et al*. 2000, Kain *et al*. 2001).

Cigarette smoking is a risk factor for both CAD and cancer but, although its mechanistic role in cancer is quite well defined (Hecht 1999), its role in CAD remains elusive. One possibility is that damage to DNA and other cellular components may have an effect on the proliferation of smooth muscle cells in the intima of the arteries, contributing to plaque formation (Bridges *et al*. 1990). This damage could be caused directly by tobacco smoke constituents binding to DNA (IARC 1994) or indirectly by oxidative stress resulting from an inflammatory response to endothelial cell damage, a process consistent with the 'response to injury' model (Ross 1993). Evidence for DNA damage and mutation in CAD is based on the observation that the smooth muscle cell population in atherosclerotic plaques shows a monoclonal origin (Murry *et al*. 1997). In addition, tobacco smoke components known to damage DNA cause plaque promotion in a number of animal models (Penn and Snyder 1996); their DNA adducts are found at high levels in vascular tissue in these models and in human tissue, including the abdominal aorta and heart (De Flora *et al*. 1997, Van Schooten *et al*. 1998, Izzotti *et al*. 2001). Another possible mechanism of action of tobacco smoke, with respect to acute myocardial infarction (AMI), is in the modulation of plaque stability. Studies have shown that the decrease in risk of AMI following cessation of smoking in susceptible individuals diminishes at a rate in excess of that which would be predicted for plaque regression (Rosenberg *et al*. 1985). This has led to the hypothesis that certain tobacco smoke components render plaque more susceptible to rupture, the latter being a key initiating event in the pathogenesis of AMI.

Many of the active components of tobacco smoke require metabolism in order to exert toxic effects. The glutathione *S*-transferases (GSTs) are a superfamily of xenobiotic-metabolizing enzymes that catalyse the conjugation of glutathione to these potentially hazardous reactive electrophilic species to detoxify them and facilitate their excretion (Ketterer 1998). As a consequence, the expression of these enzymes affects the level of cellular damage and hence can modulate disease risk. Polymorphisms in the genes for GST enzymes, in particular deletion polymorphisms in *GSTM1* and *GSTT1*, whereby homozygous deleted individuals express no functional enzyme, have previously been associated with increased adduct levels (Pastorelli *et al*. 2001) as well as an increased risk of many tobacco-related cancers (Rebbeck 1997). In a Caucasian population we observed a decreased risk of AMI in individuals with the *GSTM1* null genotype, and this effect was strongest in smokers (Wilson *et al*. 2000). The aim of the present case-control study was to examine whether GST genotype (*GSTM1* and *GSTT1*) is associated with the risk of CAD or AMI in a UK population of South Asian origin with an elevated disease incidence and different risk factors, including a reduced prevalence of cigarette smoking, compared with the indigenous Caucasian population.

## Materials and methods

### Recruitment

Over a 20 month period, 170 patients of South Asian origin, recruited at four centres in Yorkshire, UK, were admitted for routine angiography for the investigation of suspected CAD. A control group of 203 healthy South Asians, with no history of angina or AMI, were recruited from local Family Health Services Authority general practice registers. South Asians were described as subjects resident in the UK who had migrated from Pakistan, India or Bangladesh or their four grandparents were from one of these three South Asian countries. Participants were predominantly of Pakistani origin, and the majority (> 85%) were non-UK born. Clinical histories were taken for each patient and control, and all subjects gave informed consent according to a protocol approved by the United Leeds Teaching Hospitals NHS Trust and Pinderfields Health Trust Ethics Committees. Venous blood (10 ml) was taken before 9:00 a.m. following an overnight fast, and genomic DNA was extracted as described elsewhere (Mansfield *et al*. 1994).

The patient group was further subdivided into two groups on the basis of the occurrence or not of a prior AMI, as defined by World Health Organization criteria and determined from the clinical history. Results of angiography were graded as normal or single, double or triple vessel disease based on the presence of $\geq$ 50% stenosis in a major coronary artery or one of their branches as determined by ultrasonography. Smoking status was determined from interviews with the patients and controls at the time of blood sampling. Subjects were asked whether they were smokers at the time of recruitment ('current smoker') or had ever been a regular smoker ('ever smoker').

### Materials

Ethidium bromide and 6 × gel loading buffer were supplied by Sigma Chemical Co. (Louis, Missouri, USA). Ampli*Taq* Gold™ DNA polymerase, GeneAmp® 10 × PCR Buffer II, and magnesium chloride were from Perkin-Elmer Applied Biosystems (Warrington, UK). All primers, deoxynucleotide triphosphates (dNTPs), agarose and the 100 bp DNA marker ladder were supplied by Gibco BRL/Life Technologies (Paisley, UK). Polymerase chain reaction (PCR) reactions were performed on an MJ Research PTC-100 thermal cycler (MJ Research, New Jersey, USA).

### GSTT1/M1 multiplex PCR

Analysis of the *GSTT1* and *GSTM1* genes was carried out using a multiplex PCR reaction with the ubiquitous $\beta$-globin gene as an internal control, as described previously (Wilson *et al*. 2000). Briefly, a PCR reaction was carried out in a 20 µl volume containing approximately 100 ng of genomic DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP and 0.5 U of Ampli*Taq* Gold™ DNA polymerase, with 10 pmol of GSTT1-A (5′-TCT CCT TAC TGG TCC TCA CAT CTC-3′), GSTT1-B (5′-TCA CCG GAT CAT GGC CAG CA-3′), GSTM1-A (5′-GAA CTC CCT GAA AAG CTA AAG C-3′) and GSTM1-B (5′-GTT GGG CTC AAA TAT ACG GTG G-3′), and 20 pmol of $\beta$-globin-A (5′-GAA GAG CCA AGG ACA GGT AC-3′) and $\beta$-globin-B (5′-CAA CTT CAT CCA CGT TCA CC-3′) primers. The PCR conditions consisted of a 15 min pre-incubation step at 95°C, 36 cycles of 60 s at 95°C, 60 s at 60°C, 60 s at 72°C, and a final post-cycling 10 min extension step at 72°C. A 5 µl aliquot of PCR product was analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide (250 $ml^{-1}$) and the presence or absence of the *GSTT1* (460 bp) and *GSTM1* (219 bp) amplicons was determined in the presence of the control $\beta$-globin gene (268 bp).

### Statistical analysis

Results obtained for the genotypes were analysed with reference to current and past smoking status using Pearson $\chi^2$ contingency tables with Fisher's exact test and Mantel–Haenszel odds ratio estimate with 95% confidence interval. Logistic regression was used to examine the relationship between genotype and disease, incorporating other variables into the model. All analyses were performed using SPSS v9.00 (SPSS Inc.) statistical analysis software.

## Results

The descriptive data concerning the patient and control groups are presented in table 1. The mean age and sex differed significantly between the patient and control groups, with the patients being significantly older and predominantly male; mean body mass index was similar across the groups. Among the patients, 81% had occlusion $\geq$ 50% of lumen diameter in at least one of the major coronary arteries as

Table 1. Clinical and epidemiological data for the patient and control groups.

| | Controls ($n = 203$) | Patients ($n = 170$) | AMI−subgroup ($n = 89$) | AMI+subgroup ($n = 81$) |
|---|---|---|---|---|
| Age (years) | | | | |
| Mean | 45.2 | 55.5 | 54.6 | 56.5 |
| 95% CI | 43.0–47.3[a] | 53.9–57.1[a] | 52.4–56.9 | 54.8–58.8 |
| Sex (males:females) | 65:138[a] | 145:25[a] | 67:22[b] | 78:3[b] |
| Body mass index ($kg\,m^{-2}$) | | | | |
| Mean | 25.6 | 25.6 | 25.5 | 25.7 |
| 95% CI | 24.9–26.2 | 25.1–26.1 | 24.8–26.2 | 25.0–26.5 |
| Stenosis | | | | |
| None | N/A | 33 (19.4%) | 30 (33.7%)[b] | 3 (3.7%)[b] |
| Single vessel | N/A | 49 (28.8%) | 27 (30.3%)[b] | 22 (27.2%)[b] |
| Double vessel | N/A | 35 (20.6%) | 12 (13.5%)[b] | 23 (28.4%)[b] |
| Triple vessel | N/A | 53 (31.2%) | 20 (22.5%)[b] | 33 (40.7%)[b] |
| Current smokers | 13 (6.4%)[c] | 27 (15.9%)[c] | 11 (12.4%) | 16 (19.8%) |
| Ever smokers (includes current smokers) | 35 (17.2%)[a] | 78 (45.9%)[a] | 32 (36.0%)[d] | 46 (56.8%)[d] |

AMI+, patients who had had a previous myocardial infarction; AMI−, patients who had not had a previous myocardial infarction; 95% CI, 95% confidence interval; N/A, not analysed.

[a] $p < 0.001$ for patients to controls.

[b] $p < 0.001$ for AMI− to AMI+.

[c] $p \leq 0.003$ for patients to controls.

[d] $p \leq 0.01$ for AMI− to AMI+.

Table 2. Prevalence of *GSTM1* null genotype and smoking status in relation to CAD.

| | Controls | | Patients | | |
|---|---|---|---|---|---|
| | Total no. | *GSTM1* null (%) | Total no. | *GSTM1* null (%) | OR (95% CI) |
| All subjects | 203 | 52.7 | 170 | 41.2[a] | 0.63 (0.42–0.95) |
| Never smokers only | 168 | 51.2 | 92 | 37.0[b] | 0.56 (0.33–0.94) |
| Ever smokers only | 35 | 60.0 | 78 | 46.2[c] | 0.57 (0.25–1.28) |

Statistical analysis was performed using the Pearson $\chi^2$ test with Fischer's exact test and the Mantel–Haenszel common odds ratio estimate. OR, odds ratio; 95% CI, 95% confidence interval.

[a] $p = 0.029$ compared with the control group.

[b] $p = 0.037$ compared with the control group.

[c] $p = 0.223$ compared with the control group.

determined by angiography. The remaining 19% were recruited on the basis of chest pains and other indicators of CAD in the absence of stenosis. Nearly half of the patients had had a prior AMI event in their clinical history. Among this group the degree of stenosis was significantly higher than among those with no history of AMI, and they were significantly more likely to be male and have a history of cigarette smoking (table 1).

The analysis of GST genotypes showed that the *GSTM1* homozygous null genotype occurred significantly ($p = 0.029$) less frequently in the patient group than in the controls (table 2); this effect was consistent in patients irrespective of prior occurrence (42.0% null, odds ratio [OR] 0.65, 95% confidence interval [95% CI] 0.39–1.09, $p = 0.115$) or not of AMI (40.4% null, OR 0.61, 95% CI 0.37–1.01, $p = 0.058$). When subjects were stratified by smoking status, the significant association between *GSTM1* null genotype and reduced risk of disease was seen in 'never smokers' (table 2). Owing to the relatively low prevalence of smokers,

particularly among females of South Asian origin, the analysis in smokers was of limited statistical power, although the trend was similar. The frequency of *GSTM1* genotype did not vary with sex or age. This suggests that the above associations were not the result of selective mortality from other causes associated with the genotype amongst the patient group, where the mean age was higher than in controls (data not shown).

Given the marked difference in sex ratio between patients and controls, we performed the statistical analyses after stratifying by sex. The same pattern was seen in both sexes, namely a reduced prevalence of the *GSTM1* null genotype in the patient group when compared with the controls (44.0% versus 53.6% for males and 40.7% versus 50.8% for females in patients and controls, respectively). However, due to the lower numbers of subjects in the groups, the results were not statistically significant (OR 0.67, 95% CI 0.37–1.20, $p = 0.180$ in males; OR 0.68, 95% CI 0.29–1.60, $p = 0.394$ in females).

The *GSTM1* genotype frequency was entered into a logistic regression model for the development of disease in this population. Male sex and increased body mass index were the strongest risk factors, whilst the *GSTM1* null genotype was again associated with a decreased risk of developing CAD (OR 0.65, 95% CI 0.39–1.09, $p = 0.106$). In addition, the frequency of the *GSTM1* null genotype was not seen to vary with age, suggesting that the observed differences in frequencies were not simply a result of selective mortality (data not shown).

In contrast to the findings with *GSTM1*, there was no significant association between the *GSTT1* null genotype and disease (21.7% null in controls versus 20.0% null in patients, $p > 0.5$).



## Discussion

The current case-control study conducted in South Asians examined the relationship between polymorphisms in two members of the GST superfamily and the risk of CAD and AMI in patients undergoing coronary angiography. This study is important given the increased risk and different aetiology of this disease in South Asians compared with Caucasians (Cappuccio 1997, Anand *et al*. 2000).

Individuals homozygous for deletion polymorphisms in the *GSTT1* and *GSTM1* genes do not express the corresponding enzyme and have been shown to be at higher risk of certain cancers, particularly tobacco-related cancers (Rebbeck 1997). In addition, these individuals show higher levels of carcinogen-derived DNA adducts in their tissues (Ryberg *et al*. 1997, Pastorelli *et al*. 2001). Recent work indicates that the glutathione-related enzymes are expressed in the vasculature (Mezzetti *et al*. 1990, 1992, Pessah-Rasmussen *et al*. 1993) and are associated with cellular defences there (Lapenna *et al*. 1998). Thus deletion polymorphisms in one or both of these genes may be expected to have a significant effect on the levels of induced damage of DNA, proteins and lipids, and consequently disease progression. This genetic approach provides a counterpoint to existing work on the identification of DNA adducts in vascular tissue (De Flora *et al*. 1997, Izzotti *et al*. 2001, Van Schooten *et al*. 1998), levels of which have been correlated with the severity of CAD (Lapenna *et al*. 1998). The findings of the current study suggest that the *GSTM1* null deletion polymorphism is a protective factor for the development of CAD. The *GSTM1* null genotype was significantly

less represented in patients (41.2%) compared with controls (52.7%), irrespective of a patient's prior history of AMI.

Cigarette smoking appears to be a less important risk factor for CAD in South Asians than it is in Caucasian populations because of cultural differences in smoking patterns (Knight *et al*. 1993, McKeigue *et al*. 1993, Bhopal *et al*. 1999). Our data are consistent with this, showing particularly low rates of smoking in females. The low number of smokers makes the analysis of CAD risk and *GSTM1* genotype in relation to smoking status difficult to assess. However, the trend towards a decreased risk in association with the *GSTM1* null genotype was seen in 'never smokers' and 'ever smokers', suggesting that this risk factor may be acting independently of smoking in this population. It is also recognized that smoking, and other risk factors, differ in prevalence between South Asian groups (Bhopal *et al*. 1999). In the current study the subjects were predominantly of Pakistani origin and hence no subgroup analysis was performed.

Our observation is consistent with a Scandinavian study of patients with atherosclerotic occlusion of the large arteries of the limbs, which revealed that these patients were less likely to have a low-activity GSTM1 phenotype, a surrogate marker for the *GSTM1* null genotype, than control subjects (Pessah-Rasmussen *et al*. 1990). In addition, a study of polyaromatic hydrocarbon DNA adducts in atrial tissue found a tendency towards lower adduct levels in individuals null for *GSTM1*, and this effect was more marked in individuals null for both this gene and *GSTT1* (Van Schooten *et al*. 1998). As in our earlier study in Caucasians (Wilson *et al*. 2000), the above result contrasts with the observation for tobacco-related cancers that the *GSTM1* null genotype and associated lack of enzyme increases disease risk (Rebbeck 1997). These results are also contradicted by data from Li and colleagues, where the *GSTM1* null genotype was found to be a risk factor for CAD (Li *et al*. 2000) and to have no effect on the risk of lower extremity arterial disease (Li *et al*. 2001) in a case-cohort study in the USA. Clearly, an understanding of the mechanistic role of GSTM1 in CAD and AMI will be important in resolving these apparent contradictions in population-based studies.

There are a number of possible mechanistic explanations for the null genotype being protective against CAD and AMI. One is that the GSTM1 enzyme may generate a toxic metabolite that promotes atherogenesis or plaque instability, in a process similar to the ability of GSTT1 to generate a toxic byproduct as a consequence of the metabolism of halogenated hydrocarbons (Pickett and Lu 1989). A second possibility is that *GSTM1* gene deletion could lead to the upregulation of another enzyme more effective at the detoxification of a particular electrophilic metabolite that promotes atherogenesis or plaque instability. For example, it is known that cytochrome P450 1A2 activity is higher in individuals who are *GSTM1* null (MacLeod *et al*. 1997).

The association between *GSTM1* null genotype and disease risk in non-smokers suggests that the effect of the genotype may not be mediated via metabolism of a tobacco-related toxin. Rather, the enzyme substrate may come from other sources such as the diet or environmental pollution. This is in contrast to our previous UK study in Caucasians, where the risk of disease associated with the *GSTM1* genotype was strongest in smokers. It is possible that the mechanism of action of GSTM1 in disease pathogenesis is different between these two groups of subjects, or that a lifestyle factor correlated with cigarette smoking is causing the enhanced risk in Caucasians. For example, smokers tend to be more sedentary,

have a poorer diet and consume more alcohol than non-smokers (Tang *et al*. 1997). The observation in Caucasians was of a smoking-related association between *GSTM1* genotype and the risk of AMI, whereas in the South Asians the association is with CAD, with or without a prior AMI. The *GSTM1* null genotype may be primarily affecting smoking-related plaque instability in Caucasians, whereas in South Asians the effect may be related more to the formation of atherosclerotic plaques.

In conclusion, the finding of decreased CAD in individuals with the *GSTM1* null genotype supports our earlier findings in Caucasians and suggests that polymorphisms in xenobiotic-metabolizing enzymes may present a novel area of investigation in the search for genetic susceptibility to vascular disease.



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