Increased Cardiovascular Morbidity and Mortality in Type 2 Diabetes Is Associated With the Glutathione S Transferase Theta–Null Genotype
A Go-DARTS Study
Alex S.F. Doney, MRCP, PhD; Simon Lee, BSc; Graham P. Leese, FRCP, MD; Andrew D. Morris, FRCP, MD; Colin N.A. Palmer, PhD

Background—Glutathione S-transferases (GSTs) modulate oxidative stress, and variation in GST genes has been associated with cardiovascular disease risk. We prospectively determined smoking-related cardiovascular morbidity by GST genotype in a large cohort of individuals with type 2 diabetes using a population-based diabetes research database (DARTS).

Methods and Results—We performed a cohort study of 2015 individuals with type 2 diabetes. Individuals were genotyped for the Ile105Val variant of GSTP1 and the deleted variants of GSTT1 and GSTM1. Clinical characteristics, smoking status, and incidence of subsequent cardiovascular events were obtained by examining the DARTS databases. Variation in the GSTP1 and GSTM1 genes was not associated with smoking-related risk of death or cardiovascular events. There was an increase in the rate of cardiovascular events in smokers lacking the GSTT1 gene compared with smokers with the GSTT1 gene intact (hazard ratio [HR], 1.96; \( P = 0.001 \)). This excess of cardiovascular events was due to both strokes (HR, 2.7; \( P = 0.008 \)) and myocardial infarctions (HR, 1.9; \( P = 0.006 \)). The rate of death as a result of a cardiovascular event was even more markedly increased in the GSTT1-null smokers (HR, 2.7; \( P = 0.001 \)), with a 2-fold increase in myocardial infarction fatality ratio. These effects translated into an increase in overall death and a decrease in age at death. We also found that the GSTT1− genotype was associated with progression of both diabetic retinopathy and nephropathy (\( P = 0.005 \) and \( P = 0.01 \), respectively), although we found little evidence for an interaction with smoking.

Conclusions—Genetic absence of the GSTT1 enzyme is an independent and powerful predictor of premature vascular morbidity and death in individuals with type 2 diabetes. (Circulation. 2005;111:2927-2934.)

Key Words: death, sudden ■ genetics ■ myocardial infarction ■ smoking ■ stroke

Oxidative stress and inflammation are emerging as unifying pathophysiological mechanisms underlying cardiovascular disease.1-3 Type 2 diabetes is associated with a high endogenous inflammatory load and with an increased susceptibility to both macrovascular and microvascular angiopathy.4-6 Various cellular detoxification systems exist that protect against both endogenous and environmental noxious substances. In particular, the superfamily of glutathione S-transferases (GSTs) is associated with the regulation of inflammation through modulation of prostaglandin signaling pathways and oxidative stress7 and through the regulation of normal cellular physiology.8,9 The GSTs are also involved in the detoxification of many of the harmful substances found in tobacco smoke,10 a major environmental risk factor for atherosclerotic vascular disease and its clinical consequences of myocardial infarction (MI) and stroke.11,12

Human GST genes are highly polymorphic, with several variants occurring at a high frequency. These allelic variants may give rise to differential predisposition to degenerative pathological states such as cancer and arthritis.10,13

Approximately 20% of the white population is homozygous for a null variant of the gene for GSTT1-1, in which 50 kb of genomic sequence containing the entire gene is deleted (GSTT1−).14 This deletion contains only the GSTM1 gene and leaves the adjacent GSTT2 gene intact.15,16 GSTT2 expression is apparently normal in individuals with the GSTT1 deletion.17 The role of GSTT1-1 in deactivating epoxides in cigarette smoke, together with its absence in smoking-related cancers, suggests that individuals homozygous for the null allele may be at higher risk of smoking-related tissue damage resulting from an inability to neutralize toxins in cigarette smoke. Some recent studies have supported

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this hypothesis, whereas other studies have reported that the absence of the GSTT1 gene is associated with a lower risk of damage or disease. This dichotomy may be due to the ability of GSTT1-1 to activate certain carcinogens.

The GSTM1-1 enzyme detoxifies products of oxidative stress and other reactive compounds such as the polyyclic aromatic hydrocarbons. In white populations, ~50% of the population is homozygous for a 15-kb deletion allele of GSTM1. Again, this deletion removes the entire gene, and the adjoining GSTM2 and GSTM5 genes remain intact. Individuals with the GSTM1-null (GSTM1−) allele have been demonstrated to have increased susceptibility to inflammatory pathologies and an increased risk of some smoking-related cancers. Individuals who smoke and lack the GSTM1 gene develop both coronary heart disease and atherosclerosis at an increased rate.

A GSTP1 variant with a substitution in the active site of valine for isoleucine at codon 105 (Ile105Val) exists that has a reduced ability to conjugate reactive electrophiles with glutathione and may therefore sensitize cells to free radical-mediated damage. The Val105 variant has been associated with susceptibility to smoking-related cancer and cardiovascular disease.

In view of the accumulating evidence that the GST genotypes at the GSTT1, GSTM1, and GSTP1 loci influence smoking-related cardiovascular risk, we considered their association with multiple cardiovascular morbidities in relation to smoking in a large longitudinal cohort of patients with type 2 diabetes who are followed up through the population-based chronic disease management database known as Diabetes Audit and Research in Tayside Scotland (DARTS).

**Methods**

In the Tayside region of Scotland (population 400,000), detailed clinical information on all individuals with diabetes mellitus is recorded on DARTS, an electronic clinical information system. Electronic record linkage techniques have identified all people with diabetes in the population of Tayside with a sensitivity of 97%. The databases are live and continuously updated from various sources, including clinic encounters, hospital biochemistry reports, and hospital discharge data. All clinical data are recorded according to a standardized dataset, and all case records are validated by a team of local research ethics committee.

The 2015 white individuals with type 2 diabetes who took part in this study were genotyped for the GSTT1, GSTM1, and GSTP1 Ile105Val variants. DNA was prepared from blood and stored in aliquots at −20°C. The genotypes of GSTT1 and GSTM1 were determined with assays based on the presence or absence of a polymerase chain reaction amplification product and with β-globin or GSTM4 as multiplexed internal controls, as previously described. The GSTP1 genotype was assessed by use of the TaqMan-based allelic discrimination assays using the following oligonucleotides: forward primer, CCTGGTGACATGCTGAACT; reverse primer, CAACCCTGGTGAGATGCT; probe allele a (Fam/TAMRA labeled), CGCTGCAA ATACATCTCCCTCATCTACA; and probe allele b (Vic/TAMRA labeled), CGCTGCAA ATACGCTTCCCTCATCTACA. Allelic discrimination assays were performed on an Applied Biosystems 7700 sequence detection system using procedures specified by the manufacturer (Applied Biosystems).

**Macrovascular Disease Events**

All individuals were followed up until their first nonfatal or fatal cardiovascular event or death from any cause after recruitment. Nonfatal cardiovascular events were determined from both the hospital Scottish Morbidity Record and the DARTS program of nurse-facilitated validation. For fatal events, the date of death was ascertained from DARTS, with the cause of death obtained from the General Registrars Office. Classification of deaths resulting from MI was by *International Classification of Diseases*, revision 9 (ICD-9) codes 410 to 414, 4275 and 4292, and/or 799 and by ICD-10 codes I21 through I25, I50, and I56. Deaths resulting from stroke were defined as ICD codes I60 to I69 and 430 to 438. Because of a delay of ~6 months in the availability of cause of death data from the General Registrars Office, the follow-up period for adjudicated deaths was, by necessity, shorter by this period (mean follow-up time, 30 months [SD, 15.27]) than for all-cause death.

**Microvascular Disease Events**

Microvascular disease was assessed in terms of both diabetic retinopathy and nephropathy. For retinopathy, all individuals in the cohort with no current or prior record of having end-stage eye disease (defined as preretroliferative/preretroliferative retinopathy, diabetic maculopathy, or laser treatment) at the time of enrollment were followed up until the date of their first record of having end-stage eye disease or laser treatment during routine screening. Estimated creatinine clearance, used as a surrogate for nephropathy, was determined at baseline for all individuals within 1 year of enrollment by the Cockcroft-Gault formula corrected to a body surface area of 1.72 m². All individuals were followed up until the first date that their estimated creatinine clearance had reached 50% of their initial value.

**Risk Factors**

In DARTS, smoking status is recorded as current smoker, non-smoker, or ex-smoker. Because ex-smoker status was likely to be unreliablely reported and confounded because of its association with background cardiovascular events or risk, we dichotomized smoking status to simplify the modeling process. For “ever-smokers,” a current smoker and/or ex-smoker code was recorded within 1 year of recruitment. “Never-smokers” were defined as individuals who had only a nonsmoking code during this time. Glycohemoglobin, ratio of total cholesterol to HDL-cholesterol, and systolic (SBP) and diastolic (DBP) blood pressures were determined as the average of values recorded within 1 year of enrollment in the study and recorded as continuous variables. Mean arterial blood pressure (MAP) was determined as follows: MAP = [(DBP×2)/3] + SBP/3. Having been prescribed insulin or treatment for blood pressure was also determined and recorded as dichotomous variables.

**Statistical Analysis**

For this prospective survival analysis, Cox’s regression was used to determine the association of genotype with the above outcomes after recruitment until the end of the study period. The mean follow-up period overall was 36.8 months (SD, 15.9 months); for adjudicated fatal cardiovascular events, follow-up was 30 months (SD, 15.27 months). For macrovascular events, the analysis was performed in the entire group (n = 2015) and in a subcohort with no prevalent disease (n = 1624). We also modeled time to death by all causes. Initially, each genotype was tested in isolation. In the case of GSTM1 and GSTT1, a recessive model was used (i.e., complete absence of the enzyme), whereas in the case of GSTP1, both recessive and codominant models were examined. In all cases, the null allele (or reduced function genotype) was coded 1. Survival functions were adjusted subsequently for age at recruitment in all the populations.
Cox regressions, although they were removed when age at death was modeled. Smoking status was coded 1 for ever-smokers and 0 for never-smokers. The significance of the effect modification of genotype with smoking status or other risk factors was formally assessed by constructing a 2-way interaction term. For testing for the effect of smoking by genotype, a composite variable was constructed (ever-smokers genotype $H11001$, ever-smokers genotype $H11002$, never-smokers genotype $H11001$, and never-smokers genotype $H11002$) and entered as indicator variables. Fully adjusted models were also considered in which glycohemoglobin, ratio of total to HDL-cholesterol, mean arterial pressure, need for insulin and hypertension treatment, and years with diabetes were also included. STATA version 8 was used for all analyses.

### Results

Clinical and genetic characteristics of the 2015 patients taking part in the study are shown in Table 1. During the follow-up period, there were 96 fatal and 58 non-fatal MIs. There were 21 fatal and 39 nonfatal stroke events. In total, 256 deaths occurred during the monitoring period.

The frequency distributions of the different genotypes were similar to those previously reported for all 3 loci. The GSTP1 codon 105 variant was present in Hardy-Weinberg equilibrium; it was not assessed for the GSTM1 and GSTT1 variants because the frequency of the heterozygote null is not determined by current genotyping techniques.

In our initial analysis, we found that absence of the GSTT1 genotype (GSTT1$^-$ genotype) was associated with an increase risk of cardiovascular events (Table 2). There was no evidence that GSTM1 and GSTP1 variants were associated with cardiovascular events in this population (Table 2). Because further analysis provided no evidence that these variants modulated the risk associated with GSTT1, they were not considered further.

We then sought to determine the influence of smoking in the observed association of GSTT1$^-$ genotype on cardiovascular outcome and so included smoking status in the model. We found a significant interaction of the GSTT1$^-$ genotype with smoking status (hazard ratio [HR], 2.2; 95% CI, 1.1 to 4.3; $P=0.026$); therefore, we proceeded to consider smokers and nonsmokers separately (Table 3), including both years with type 2 diabetes and age at enrollment in the study. We found that among nonsmokers the GSTT1$^-$ genotype had no increase in hazard of cardiovascular events (HR, 0.91; 95% CI, 0.52 to 1.59; $P=0.74$) compared with GSTT1$^+$ genotypes. Conversely, among smokers, individuals who were GSTT1$^-$ were at significantly higher risk (HR, 1.96; 95% CI, 1.33 to 2.91; $P=0.001$) compared with those with GSTT1$^+$. These data are depicted as survival curves in Figure 1A. As expected, individuals with the GSTT1$^+$ genotype who smoke

### Table 1. Population Characteristics and Genotype Frequencies

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% or Mean (SD)</th>
</tr>
</thead>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1058</td>
<td>52.5</td>
</tr>
<tr>
<td>F</td>
<td>957</td>
<td>47.5</td>
</tr>
<tr>
<td>Total</td>
<td>2015</td>
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</tr>
<tr>
<td>Mean age at genotyping, y</td>
<td>64.4 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Mean time with diabetes at genotyping, y</td>
<td>7.9 (6.6)</td>
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<tr>
<td>Prevalent CVD</td>
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<tr>
<td>Yes</td>
<td>391</td>
<td>19.4</td>
</tr>
<tr>
<td>No</td>
<td>1624</td>
<td>80.6</td>
</tr>
<tr>
<td>Ever-smoker</td>
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<tr>
<td>Yes</td>
<td>925</td>
<td>48.9</td>
</tr>
<tr>
<td>No</td>
<td>965</td>
<td>51.1</td>
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<td>GSTT1</td>
<td></td>
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<tr>
<td>+</td>
<td>1589</td>
<td>79.7</td>
</tr>
<tr>
<td>–</td>
<td>405</td>
<td>20.3</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
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<tr>
<td>+</td>
<td>840</td>
<td>44.2</td>
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<td>–</td>
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<td>55.8</td>
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<td>GSTP1: codon 105</td>
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<tr>
<td>Ile/Ile</td>
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<td>41.8</td>
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<tr>
<td>Ile/Val</td>
<td>895</td>
<td>44.4</td>
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<tr>
<td>Val/Val</td>
<td>278</td>
<td>13.8</td>
</tr>
</tbody>
</table>

### Table 2. HRs for Stroke, MI, and Cardiovascular Death by GST Genotype Corrected for Age

<table>
<thead>
<tr>
<th>GSTT1</th>
<th>Events/Those at Risk</th>
<th>HR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTT1$^+$</td>
<td>190/1994</td>
<td>1.47</td>
<td>0.017</td>
<td>1.07–2.02</td>
</tr>
<tr>
<td>GSTT1$^-$</td>
<td>179/1899</td>
<td>1.08</td>
<td>0.59</td>
<td>0.81–1.46</td>
</tr>
<tr>
<td>GSTP1 codominant</td>
<td>192/2015</td>
<td>1.04</td>
<td>0.84</td>
<td>0.70–1.57</td>
</tr>
<tr>
<td>GSTP1 recessive</td>
<td>192/2015</td>
<td>1.07</td>
<td>0.74</td>
<td>0.72–1.60</td>
</tr>
</tbody>
</table>

### Table 3. Interaction of Smoking Status With GSTT1 Genotype for Hazard of Cardiovascular Events, Including Death

<table>
<thead>
<tr>
<th>GSTT1 $^-$</th>
<th>Events/Those at Risk</th>
<th>Age Corrected</th>
<th>Fully Corrected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>never-smokers</td>
<td>135/1563</td>
<td>1.46</td>
<td>0.029</td>
</tr>
<tr>
<td>ever-smokers</td>
<td>74/1006</td>
<td>0.91</td>
<td>0.744</td>
</tr>
<tr>
<td>never-smokers</td>
<td>95/977</td>
<td>2.88</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>ever-smokers vs GSTT1$^+$ ever-smokers</td>
<td>114/956</td>
<td>1.96</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Corrected for age at enrollment, years since diagnosis with diabetes, glycohemoglobin, insulin treatment, mean arterial blood pressure, hypertensive treatment, and log10 ratio of total to HDL cholesterol.

†Referent category.
were at a significantly higher risk compared with nonsmokers (HR, 1.46; 95% CI, 1.04 to 1.06; P=0.029). Therefore, GSTT1− smokers had a highly significant HR of 2.88 (P=0.0001) compared with GSTT1+ smokers.

There was no difference in other risk factors (systolic blood pressure, ratio of total to HDL cholesterol, glycohemoglobin, or need for insulin or antihypertensive therapy) between GSTT1 genotypes (data not shown). When we included these parameters, together with age at enrollment and years with type 2 diabetes, in a fully adjusted model, the impact of the GSTT genotype on outcome was largely unaffected (Table 3). In this multivariate model, only age at enrollment (HR, 1.06 per year; 95% CI, 1.05 to 1.08; P<0.0001), requirement for insulin therapy (HR, 2.1; 95% CI, 1.40 to 3.0; P<0.0001), and log ratio of total to HDL cholesterol (HR, 2.8; 95% CI, 1.00 to 7.5; P=0.049) were also significant predictors of outcome. On formal testing, there was no evidence of effect modification of any of these variables with genotype.

We found a similar increased risk of GSTT1− genotype compared with GSTT1+ when we considered stroke (HR, 2.7; 95% CI, 1.3 to 5.6; P=0.008) and MI separately (HR, 1.9; 95% CI, 1.2 to 2.9; P=0.006) among smoke-exposed individuals. We also investigated the association of GSTT1− on incident cardiovascular events in the 80% of the population that did not have any previous cardiovascular events and found that the hazard associated with the GSTT− genotype was even greater in this analysis (Figure 1B). When we considered cardiovascular death alone as an end point, the risk associated with GSTT1− compared with GSTT1+ in smokers was found to be amplified (Figure 1C). The increased risk of cardiovascular death compared with cardiovascular nonfatal cardiovascular events was due an decreased rate of survival of MI, with the GSTT1− smokers being 2.2-fold more likely to die if they had an MI compared with the GSTT1+ smokers (P=0.042, Fisher’s exact test). Therefore the GSTT1− smokers have an increased chance of dying after an MI, in addition to an increased chance of an incident MI. We then wanted to determine whether the observed increase in cardiovascular risk was translating to an overall impact on mortality in the diabetic smoking population. Therefore, we modeled all-cause death using all the observed deaths and observed a similar relationship, with GSTT1− ever-smokers having a hazard ratio of 1.7 (P=0.007) compared with GSTT1+ ever-smokers (Figure 2A). To visualize the impact of these findings on the actual age at death in this population, we modeled age at death in ever-smokers (Figure 2B) and found that there was a significant hazard of dying at an earlier age in ever-smokers with the GSTT1− genotype (HR, 1.7; P=0.005).

The GSTT1− genotype did not confer risk of cardiovascular events in individuals who had never smoked (summarized in Figure 3).

Because we had found an association of GSTT1 genotype with macrovascular disease, we subsequently explored the possibility that it may also be associated with more general vascular disease. We therefore examined the association of GSTT1 genotype on 2 major forms of microvascular disease affecting diabetic populations, namely diabetic retinopathy and nephropathy. We found that the GSTT1− genotype was again associated with an increased risk for both of these pathologies (Table 4 and Figure 4). Again, this association remained significant even after correction for conventional risk factors.

We found that both age at enrollment and years with diabetes were both significant predictors of nephropathy (HR, 1.02 per year; 95% CI, 1.01 to 1.04; P=0.008, and HR, 1.03;
95% CI, 1.01 to 1.06; \( P = 0.007 \), respectively). Although both smoking status (HR, 1.43; 95% CI, 1.02 to 2.00; \( P = 0.036 \)) and mean arterial blood pressure (HR, 1.02 per 1 mm Hg; 95% CI, 1.01 to 1.03; \( P = 0.004 \)) were also significantly associated with increased risk, we found no interaction of either of these parameters with genotype.

For diabetic retinopathy, we found that years with diabetes (HR, 1.06 per year; 95% CI, 1.03 to 1.09; \( P < 0.0001 \)), glycohemoglobin (HR, 1.31 per percent; 95% CI, 1.07 to 1.61; \( P = 0.009 \)), and a history of requiring insulin treatment (HR, 1.91; 95% CI, 1.07 to 3.45; \( P = 0.03 \)) were significantly associated with increased risk and that these parameters did not interact with the GSTT1 genotype.

### Discussion

The family of GTSs plays an important role in detoxifying both endogenous and environmental toxins. Common variants of their genes may therefore have health implications for individuals subjected to a higher exposure to these toxins. We have demonstrated in this large type 2 diabetic population from Scotland that the GSTT1\(_{-} \) genotype, in which individuals completely lack GSTT1 enzyme activity, has a powerful influence on smoking-related atherosclerotic macrovascular risk. Individuals homozygous for the GSTT1\(_{-} \) allele who smoke are at a 2-fold greater risk of a cardiovascular event or death from any cause compared with individuals who also smoke but possess GSTT1 activity. They also have an even higher risk of death resulting from a cardiovascular cause. We have also shown for the first time that the increased risk of macrovascular atheroembolic events is independently due to an increase in both the hazard of stroke and MI. The potential clinical importance of these findings is underlined by the fact that we have also been able to demonstrate that these individuals die at an earlier age. Finally, we have demonstrated that GSTT1\(_{-} \) individuals have a more generalized vasculopathy, with an increased risk of progression of both diabetic nephropathy and sight-threatening retinopathy, and this association is not influenced by smoking status. Because of the constraints of the genotypic assay in which we detected only the presence or absence of the GSTT1 gene, we were unable to investigate the potentially important question of gene dosage.

Although several studies have demonstrated an association between GST polymorphisms and smoking-related cardiovascular risk in coronary, peripheral vascular, and free living populations, we are not aware of any previous studies that have considered this association in a population.

### Table 4. Association of GSTT1 Genotype With Diabetic Microangiopathy

<table>
<thead>
<tr>
<th>Events/Those at Risk</th>
<th>Uncorrected</th>
<th>Fully Corrected*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>( P )</td>
</tr>
<tr>
<td>Decline of ECC to 50%</td>
<td>156/1927</td>
<td>1.58</td>
</tr>
<tr>
<td>Progression of retinopathy</td>
<td>76/1829</td>
<td>1.98</td>
</tr>
</tbody>
</table>

ECC indicates estimated creatinine clearance.

*Corrected for age, years with diabetes, glycohemoglobin, insulin treatment, mean arterial blood pressure, hypertensive treatment, ratio of total to HDL cholesterol, and smoking status.
with type 2 diabetes. Furthermore, no previous studies have considered their influence on diabetic microvascular disease. Interestingly, we found little evidence that GSTM1 or GSTP1 genotype influenced cardiovascular risk in this population, nor did we find any convincing evidence from multivariate analysis that the presence of these significantly modulated the impact of GSTT1–. Previous studies of the null polymorphism of GSTM1, however, have demonstrated an association with cardiovascular disease, indicating that the null allele either is protective or confers increased risk\(^{18–21,27–29}\) and furthermore that this association is influenced by GSTT1 genotype. \(^{18,19}\) The absence of an observed association in this study may reflect differences in this type 2 diabetic population compared with populations in other studies, which were nondiabetic and tended to be younger. For instance, the average age of this subgroup of the DARTS population is \(\approx 65\) years. We have previously shown that GST-null genotypes are associated with premature carotid artery disease among systemic sclerosis patients as young as 45 years of age, with the association being attenuated in older populations. \(^{32}\) It is therefore possible that the influence of the GSTM1 polymorphism is being obscured in this relatively older diabetic population. On the other hand, there was no evidence of a distortion in GSTM1 allele frequency between individuals enrolled in this population at a younger age compared with those enrolled at an older age. Currently, we have insufficient incident disease in the individuals recruited between the ages of 35 to 60, so we cannot yet rule out the involvement of either GSTM1 or GSTP1 in cardiovascular risk in this age range.

As demonstrated in Table 1, \(\approx 20\%\) of individuals at the time of enrollment had previous MI or stroke, and even greater numbers had angina and hypertension. We therefore modeled time to first cardiovascular event from genotyping both in the absence of or regardless of a documented previous event and found the population free of prevalent events demonstrated an even stronger genotype-associated risk. This is probably a result of the influence of both survival bias and the fact that individuals with prevalent cardiovascular disease are likely to be taking greater numbers of cardiovascular protective drugs compared with the prevalent event-free individuals.

The metabolism of tobacco smoke–derived toxins, together with endogenously derived reactive oxygen species, is complex, involving multiple pathways, any of which may be more or less significant, depending on the background toxic burden. In this study, we considered individuals as ever-smokers if they had a history of previous exposure to tobacco smoke regardless of whether they were recorded as nonsmokers at the time of entry into the study. This approach was pragmatic, being constrained by the nature of smoking information in DARTS and the fact that ex-smoker status is likely to be unreliable ascertained and confounded by association with background cardiovascular risk. Therefore, we have not been able to investigate the important issue of the relationship of genotype with extent of exposure to tobacco smoke.

GST deletions have been shown to associate with chronic tissue damage in inflammatory disease regardless of smoking status. \(^{7,10,38,39}\) Our demonstration that the GSTT1– genotype is associated with progression of both nephropathy and diabetic retinopathy in this population with type 2 diabetes is consistent with the role of oxidative stress in the pathophysiology of these conditions\(^{40–42}\) and indicates a role for this genotype in predisposing to a generalized vasculopathy. Although we found that smoking status was significantly associated with an increased risk of nephropathy, which is consistent with previous reports,\(^{53–45}\) there was no evidence for an interaction of genotype with smoking in this case. Furthermore, we found no evidence that smoking influenced the risk of progression of diabetic retinopathy, which again is consistent with previous findings.\(^{46}\) Differences in the relative importance of genetic and conventional risk factors, together with their interaction, in determining microvascular and macrovascular risk in this population highlight the differences in the pathobiology of atherosclerosis and its associated atherothrombotic sequelae and diabetic microvascular disease. In particular, in this study, we have considered acute clinical atherothrombotic events (stroke and MI) rather than the underlying atherosclerosis. This approach contrasts with our study of both retinopathy and nephropathy, which considered time to a predetermined pathological phenotype,
indicating progression of disease (sight-threatening retinopathy or photocoagulation) and a 50% reduction in estimated creatinine clearance). It is likely, given previous studies, that the GSTT1− variant is associated with background atherosclerotic disease in this diabetic population. Because smoking is known to increase the background atherosclerotic burden and to provoke acute atherothrombotic events, it may be that the observed increased hazard of acute atherothrombotic events observed only in GSTT1− smokers may arise from an increased prothrombotic state on exposure to tobacco smoke. Although it might be expected both from biological rationale and previous evidence that antioxidants would protect from atherosclerotic vascular disease, this has not been borne out by large trials. Given the effect of smoking-related toxins in an environment lacking GSTT1 activity, it is possible to speculate that the use of antioxidants may be of value only to individuals with a particular genotype under a high oxidant load. A previous study, for example, has demonstrated that individuals who smoked and were GSTM1− but were taking vitamin E had half the rate of increase in carotid intima-media thickness compared with GSTM1-null smokers not taking vitamin E. Therefore, although antioxidant supplementation has not worked in current trials, the genetic evidence that the glutathione defense mechanism is important in protecting smokers from disease would suggest that trials based on genotype selection might reveal a hitherto obscured benefit of antioxidant vitamins. Investigation of gene environment interactions in this way may improve targeting of therapy to individuals who are more susceptible to disease.

Acknowledgments

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References


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