Atherosclerosis and thrombosis are the 2 major contributing mechanisms in coronary artery disease (CAD), a major health problem and leading cause of death worldwide. The formation of atherosclerotic lesions is augmented by elevated levels of reactive oxygen species (ROS).  

Endothelial cell injury is initiated by increased shear stress, caused by the combination of high blood viscosity, hypertension and vasoconstriction which may be induced by smoking. The toxic chemicals present in tobacco smoke affect the endothelium and enhance smooth-muscle cell proliferation by inducing platelet adherence to damaged cells. Nicotine is a strong activator of the sympathetic nervous system, which releases catecholamines that interfere with haemodynamics, resulting in increased myocardial oxygen demand. Cigarette smoke contains a complex mixture of 4,000 chemical species and plentiful oxygen-, nitrogen- and carbon-centred free radicals which increase ROS generation and promote oxidative damage.  

Cells produce ROS through normal metabolic processes and disruption of the mitochondrial electron transport chain. Cell-intrinsic mechanisms, such as the glutathione (GSH) antioxidant defence system, act to combat ROS. Disturbances in the endogenous antioxidant defence mechanism or ROS overproduction, however, lead to accumulation and availability of free radicals which induce oxidative damage to biomolecules by lipid peroxidation, protein nitration and DNA adduct formation.

Cellular detoxification systems protect against endo- and exogenous harmful substances. Of particular interest is the super-family of glutathione S-transferases (GSTs), which modulate prostaglandin signalling pathways and oxidative stress. GSTs function to detoxify electrophilic toxicants, including those found in cigarette smoke, by facilitating conjugation to GSH. However, GST function is influenced by genotypic differences from single nucleotide polymorphisms (SNPs).  

GST genes contain several polymorphic variants occurring at high frequency. In the GSTP1 A_{105}/G_{105} variant, a single nucleotide change from adenine (A) to guanine (G) at codon 105 results in an isoleucine (Ile) to valine (Val) amino acid change. In the GSTM1 null (0/0) polymorphism, complete deletion of the gene encoding this isoform occurs at higher frequencies in CAD patients compared with the control group (36% vs 18% and 65% vs 48%, respectively).  

A significant association with CAD was observed in GSTM1 0/0 (odds ratio (OR)=2.593; 95% confidence interval (CI) 1.353 - 4.971; p=0.0043) and GSTP1 A_{105}/A_{105} (OR=0.601; 95% CI 0.3803 - 0.9503; p=0.0377). We found a significant association between smoking and CAD; the presence of either of the respective genotypes together with smoking increased the CAD risk (GSTP1 A_{105} relative risk (RR)=1.382; 95% CI 0.958 - 1.994; p=0.0987 and GSTM1 null RR=1.725; 95% CI 1.044 - 2.851; p=0.0221).

Conclusion. Our findings support the association of genotypes GSTM1 0/0 and GSTP1 A_{105}/A_{105} and smoking with CAD.  

Methods

A total of 102 young Indian CAD patients and 100 age-, race- and sex-matched controls were enrolled following institutional ethical approval (BE154/010). A full pathology report was compiled. The inclusion criteria for CAD patients were: Indian ancestry and unrelated, adults aged <45 years, and stable CAD confirmed at angiography. The exclusion criteria for controls included an acute coronary syndrome/vascularisation procedure in the preceding 3 months, chronic renal or liver disease, malignancy and known active inflammatory or infectious disease.

Genomic DNA was extracted from whole blood. Cells were transferred to 500 μl lysis buffer (0.5% sodium dodecyl sulphate (SDS), 150 mM NaCl, 10 mM ethylenediaminetetra-acetic acid (EDTA), 10 mM Tris-HCl (pH 8.0)). RNase A (100 µg/ml; DNase-free) was added and the mixture incubated (37°C, 1 h). Subsequently, proteinase K (200 µg/ml) was added and incubated (3 h, 50°C) and a 0.1% volume 5 mM potassium acetate was added before centrifugation (5000 x g, 10 min). Supernatants containing genomic DNA were transferred to fresh tubes and extracted with 100% isopropanol and washed with 70% ethanol. DNA samples were solubilised in 10 mM Tris and 0.1 mM EDTA (pH 7.4, 4°C). DNA concentration was determined spectrophotometrically.

Differential polymerase chain reaction (PCR) was performed to assess the GSTM1 polymorphism: 268 base pair (bp) (β-globin) and 215 bp (GSTP1) PCR products were amplified using 30 pmol of primers for the GSTM1 gene and 10 pmol primers for the β-globin gene in a 25 μl reaction (200 μM of each deoxyribonucleotide (dNTP), 3.3 mM MgCl₂, 1x Green GoTaq DNA polymerase, (Promega), 100 ng genomic DNA template). Primer sequences: GSTM1-Fwd 5'-GAAGAGCCAAGGACAGGTAC-3'; β-globin-Fwd 5'-GAACCTCCTGAAGAAGCTAAGC-3'; GSTM1-Rev 5'-GTTGGGCTCAAATATACGGTGG-3'; β-globin-Rev 5'-AAAGGAGGAGCAGGAGTTAC-3'. Following denaturation (96°C, 5 min), amplification was carried out with 25 cycles of denaturation (96°C, 30 sec), annealing (57°C, 30 sec) and extension (72°C, 30 sec), followed by a final extension (72°C, 5 min). Amplification products were electrophoresed on an agarose gel (4%, 1x Tris-buffered saline (TBS)). Following visualisation of bands, the presence of a single 176 bp PCR product was amplified using 15 pmol of each primer.

Statistical analyses were performed with GraphPad Prism software (version 5.0).

Results

Among the clinical parameters assessed between the groups, body mass index (BMI), hypertension, diabetes, triglycerides, fasting glucose and HbA₁c were higher in patients than in controls (Table 1). Single nucleotide changes at codon 105 of the GSTP1 gene were investigated using RFLP-PCR. The genotype frequencies observed did not deviate from those predicted by Hardy-Weinberg statistics (GSTP1: p=0.294 in CAD patients, p=0.413 in controls; GSTM1 p=0.083 in CAD patients, p=0.64 in controls; chi-square test).

A significant skew towards frequency of the A₁₀⁵/G₁₀⁵ genotype was observed in CAD patients compared with the controls. In the control group, the homozygous A₁₀⁵/A₁₀⁵ and heterozygous A₁₀⁵/G₁₀⁵ genotypes were observed at frequencies of 48% and 45%, respectively. The frequency of the A₁₀⁵/G₁₀⁵ genotype was significantly higher in CAD patients (65% v. 48%, respectively; odds ratio (OR)=0.6011, 95% confidence interval (CI) 0.3803 - 0.9503, p=0.0377; Table 2). GSTM1 0/0 was also significantly more frequent in CAD patients (36% v. 18%; OR=2.593, 95% CI 0.2952 - 1.088; Table 4) compared with +/+ and +/0 genotypes.

A higher frequency of the GSTP1 A₁₀⁵ and GSTM1 null alleles was observed in patients; therefore, we investigated whether there were genotypic differences in relative CAD risk in respect of these loci. Our findings suggest that the presence of either of these genotypes confers a significant CAD risk (Table 2). We stratified the groups according to smoking history: the number of smokers in the patient group was much higher than in the control, with a significant association between smoking and CAD (p<0.0001, OR=0.2245, 95% CI 0.1062 - 0.4746).

Most smokers presented with GSTP1 A₁₀⁵/A₁₀⁵ (p=0.0987, OR=0.5667, 95% CI 0.2952 - 1.088; Table 4) and GSTM1 0/0 (p=0.0221, OR=2.386 95% CI 1.37 - 5.009; Table 4). The higher relative risk of CAD associated with these genotypes is increased with smoking.

Discussion

The INTERHEART study ranked smoking as the second highest risk factor for myocardial infarction (OR=2.87, 99% CI). The Systemic Coronary Risk Evaluation project estimated the 10-year fatal cardiovascular risk to be twice as high for smokers v. non smokers for any given age, systolic BP and cholesterol level. The number of smokers worldwide is expected to reach 1.7 billion by 2025. With current trends, smoking-related deaths are expected to rise from 4.8 million in 2000 to 8 million in 2030.

Endothelial cell damage in vasculature is caused by changes in haemodynamics and oxidative stress, leading to thrombosis and atherosclerosis. The continuous cycle of inflammation from atherosclerotic plaques and arterial wall lesions contributes to elevated levels of C-reactive protein in CAD. The low-grade inflammation, recruitment and activation of leucocytes to the atherosclerotic lesion plays an important role in ROS generation in an attempt to circumvent the spread of foreign material, while simultaneously exposing biomolecules to oxidative stress.

Our data show an association of wild-type GSTP1 A₁₀⁵/A₁₀⁵ and GSTM1 0/0 with CAD. The presence of allele GSTP1 G₁₀⁵ in the healthy control patients signals a possible role of this polymorphism in enhancing the efficacy of antioxidant mechanisms. GST acts in circumventing the spread of foreign material, while simultaneously exposing biomolecules to oxidative stress.
GSTM1 0/0 results in complete absence of the enzyme isoform, which affects detoxification capacity. The strong association of this genotype in CAD patients indicates a key role in disease pathogenesis. The association between smoking and CAD in this cohort is in agreement with previous studies. The high percentage of patients who smoked and presented with GSTP1 A105/A105 and GSTM1 0/0 supports the hypothesis that GSTs play an important role in CAD.

Study limitations included sample size, lack of exclusion criteria based on body mass index (BMI), hypertension and diabetes.

Conclusion
This study provides evidence that the GSTP1 A105 allele and the GSTM1 null genotype are associated with CAD in young South Africans of Indian ancestry.

Acknowledgement. A Phulukdaree acknowledges an NRF-DAAD Scholarship.

References
Table 4. Summary of smokers stratified by genotypes for CAD risk identification

<table>
<thead>
<tr>
<th></th>
<th>Control n (%)</th>
<th>CAD patients n (%)</th>
<th>p</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smokers</td>
<td>35 (35)</td>
<td>11 (10)</td>
<td>&lt;0.0001</td>
<td>1.826</td>
</tr>
<tr>
<td>Smokers/ex-smokers</td>
<td>65 (65)</td>
<td>91 (90)</td>
<td>0.0987</td>
<td>1.382</td>
</tr>
<tr>
<td>GSTP1 A105/G105</td>
<td>31 (31)</td>
<td>31 (30)</td>
<td>0.0221</td>
<td>1.725</td>
</tr>
<tr>
<td>GSTM1 0/0</td>
<td>13 (13)</td>
<td>34 (33)</td>
<td>0.0221</td>
<td>1.725</td>
</tr>
</tbody>
</table>

RR = relative risk; CI = confidence interval.


Accepted 15 March 2012.