Lack of association between antioxidant gene polymorphisms and progressive massive fibrosis in coal miners

B Yucseloy, V J Johnson, M L Kashon, K Fluharty, V Vallyathan, M L Luster

Background: Oxidative stress plays a major role in the pathogenesis of interstitial lung diseases. The antioxidant enzymes glutathione S-transferases (GST) and manganese superoxide dismutase (MnSOD) are important components of lung defence against oxidative stress, and polymorphisms in the genes which regulate their expression may represent important disease modifiers.

Methods: A matched case-control study was conducted to determine the influence of the GSTP1, GSTT1 and MnSOD polymorphisms on susceptibility to progressive massive fibrosis (PMF). Seven hundred ex-coal miners were included in the study; 350 were classified as PMF cases while 350 with a similar underground mining tenure but no clinical or histological evidence of lung disease served as controls. Genotype analysis was performed on genomic DNA, using a 5' nuclease PCR assay.

Results: None of the individual investigated polymorphisms and two-way gene–gene interactions had a statistically significant association with PMF.

Conclusion: The results of this study suggest that polymorphic genotypes within the GST gene cluster and MnSOD do not affect individual susceptibility to PMF.
measured more than 1 cm, thus meeting the criteria of classical PMF. Control miners with similar exposure histories but without apparent pulmonary disease or inflammation were matched for age and years of mining tenure.

**DNA preparation and genotyping**

Genomic DNA was prepared from formalin fixed, paraffin embedded lung tissue blocks following microwave deparaffinisation using a DNA isolation kit (Qiagen, Chatsworth, CA, USA), according to the manufacturer’s instructions. Genotype analysis was performed on genomic DNA using a 5' nuclease PCR assay (Taqman®). Primers and probes were designed using Assay-by-Design service (PE Applied Biosystems, Foster City, CA, USA). Table 1 shows the primer and probe sequences for each polymorphism. PCR amplification was performed in a volume of 25 µl containing 10 ng genomic DNA, 12.5 µl 2X Taqman Universal Master Mix (PE Applied Biosystems, Foster City, CA, USA), 200 nM probe, and 900 nM primer. Cycling conditions were 50 °C for 2 min, 95°C for 10 min, followed by 50 cycles at 92°C for 30 s and 60°C for 1 min. Amplification was performed using an iCycler thermal cycler. GAPDH probe was used as internal control for the deletion polymorphism of GSTT1. GAPDH probe was used as internal control for the deletion polymorphism of GSTT1. 

**Statistical analysis**

Individuals from the PMF population (n = 350) were matched with individuals free of PMF for age and years of underground mining. Matching was performed using a macro-program (Match) written in SAS (Cary, NC, USA) by Kosanke and Bergstrahl and made available through the Division of Biostatistics at the Mayo Clinic (Rochester, MN, USA).

Analyses for associations were performed using conditional logistic regression using STATA version 8.0 (Stata Corporation, College Station, TX, USA). This analysis uses only complete matched pairs in determining an estimate of the association. As unsuccessful genotyping of subjects did not occur in the same individuals for every gene, each analysis does not necessarily have the same sample size. However, the reported frequencies for each genotype are based on all successfully genotyped samples. The effects of smoking status and pack-years were examined to determine the influence of smoking on potential associations. However, as no substantive differences in the results occurred, unadjusted odds ratios are reported. Analyses were extended to examine all possible two-way gene–gene interactions.

### RESULTS

A summary of the demographic characteristics of the study population is presented in table 2.

The genotype frequencies for the polymorphisms are shown in table 3. No significant deviation of genotype frequencies from Hardy-Weinberg equilibrium was noted in either the control or PMF groups. The allele frequencies for GSTP1, GSTT1 and MnSOD in the control population were similar to those determined in other studies involving white populations. This study failed to find differences in the genotype frequencies or the allelic frequencies between PMF cases and controls for the GSTT1, GSTP1 or MnSOD (table 3). Furthermore, no significant gene–gene interactions were detected (data not shown).

### DISCUSSION

Reactive oxygen species play a central role in the pathogenesis of a variety of interstitial lung diseases. Both experimental and human studies have suggested that exposure to mineral dusts generate ROS which are implicated directly in the pathogenesis of CWP and PMF. The ROS generating capacity of macrophages and plasma glutathione peroxidase activity of subjects exposed to coal dust also revealed a correlation with disease severity. Although several studies have supported a role for imbalances in ROS and antioxidant gene polymorphisms in the pathogenesis of interstitial lung disease, the results of these studies have been inconsistent.

### Table 2 Mean (SD) characteristics of the participants in the study groups

<table>
<thead>
<tr>
<th></th>
<th>No of cases</th>
<th>Age</th>
<th>Years of smoking</th>
<th>Years of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>350</td>
<td>70.0 (8.8)</td>
<td>16.6 (18.1)</td>
<td>33.1 (11.6)</td>
</tr>
<tr>
<td>PMF</td>
<td>350</td>
<td>70.1 (8.9)</td>
<td>16.0 (18.8)</td>
<td>33.1 (11.7)</td>
</tr>
</tbody>
</table>
antioxidant/oxidant systems in the incidence, prevalence, and severity of respiratory diseases, only limited studies have evaluated the association between antioxidant gene polymorphisms and pulmonary fibrosis and, to our knowledge, this is the first case-control study to investigate possible associations between PMF and antioxidant gene polymorphisms.

None of the investigated polymorphisms had a statistically significant effect on PMF when studied individually or in two-gene combinations. This result supports and extends findings from a study in which no associations were found in the genotype frequency of *MnSOD* or *GSTT1* between Chinese miners with CWP and miners without CWP. In a multifactorial disease such as PMF, it is likely that genetic susceptibility is dependent on the effect of multiple gene polymorphisms acting in combination with exposure. Previous reports have shown that combinations of GST polymorphisms—rather than individual genotypes—have associations with lung cancer and a rapid decline in lung function. To test this in our population, gene–gene interactions were analysed but no significant association with disease was found.

A crucial problem in case-control studies has been small sample size resulting in insufficient statistical power to determine whether an association between a variant and a disease exists. The present matched case-control study had sufficient power to detect an OR of 1.8 if it existed. Based on this, the present study is able to report no association between GST and *MnSOD* genotypes and PMF with sufficient statistical power. Although our study design is not appropriate for such evaluation due to presence of only severe cases, inter-individual differences in antioxidant production may influence disease progression by acting as disease modifiers. Increased risk could depend also on genetic variants involved in the modulation of extracellular matrix or fibrogenesis which are not necessarily affected by a GST expression such as platelet derived growth factor, matrix metalloproteinases, fibronectin, and transforming growth factor-β.

In conclusion, none of the individual GST or *MnSOD* genotypes or two-way gene–gene interactions had a statistically significant association with PMF. Although we failed to show an association, our findings do not exclude the possibility that, together with other genetic and environmental impairments, antioxidant genes may play a significant role in the severity of inflammatory or fibrotic lung diseases.

**ACKNOWLEDGEMENTS**

The authors thank Patsy A Willard for excellent technical assistance in the preparation of tissues and Drs Michael Andrew and Omur C Elci for their excellent review of the manuscript during preparation.

### Table 3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PMF n (%)</th>
<th>Controls n (%)</th>
<th>OR (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>244 (80.3)</td>
<td>255 (77.3)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Null</td>
<td>60 (19.7)</td>
<td>75 (22.7)</td>
<td>0.84 (0.54 to 1.30)</td>
</tr>
<tr>
<td>GSTP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>129 (44.2)</td>
<td>142 (44.1)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>118 (40.4)</td>
<td>134 (41.6)</td>
<td>0.93 (0.64 to 1.32)</td>
</tr>
<tr>
<td>Val/Val</td>
<td>45 (15.4)</td>
<td>46 (14.3)</td>
<td>0.99 (0.60 to 1.62)</td>
</tr>
<tr>
<td>MnSOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>46 (23.2)</td>
<td>72 (29.1)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>AV</td>
<td>138 (46.5)</td>
<td>168 (51.2)</td>
<td>0.85 (0.55 to 1.29)</td>
</tr>
<tr>
<td>VV</td>
<td>90 (30.3)</td>
<td>88 (26.8)</td>
<td>0.94 (0.59 to 1.51)</td>
</tr>
</tbody>
</table>

*Frequencies are based on all samples with successful genotyping.

**REFERENCES**


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LUNG ALERT

A new class of antitubercular drugs: the diarylquinolines


Over three million people die from tuberculosis (TB) every year, and drug resistance is an increasing problem. This paper reports the development of a promising new anti-TB agent, R207910, a diarylquinoline. It acts as an inhibitor of mycobacterial ATP synthase, a different mechanism of action from any other anti-TB drug. This minimises the potential for cross-resistance and suggests that R207910 may prove effective in the treatment of MDR-TB, as demonstrated by the authors in vitro. It is specific in activity to mycobacteria, including the opportunistic species MAC and M. kansasii, with in vivo bactericidal activity against M. tuberculosis in a murine model.

Animal and human pharmacokinetic and pharmacodynamic studies show that R207910 has a long plasma half-life, good tissue penetration, and potential for a dose regimen of less than five doses per week, making it ideal for DOT. Human studies have so far shown good tolerability to a treatment dose with no major adverse events.

The authors have shown that R207910 is an effective antitubercular agent with pharmacokinetic properties favouring good treatment adherence. It remains to be seen if these results are confirmed in clinical trials.

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