Association between the CYP1A1 gene polymorphism and susceptibility to emphysema and lung cancer

A M Cantlay, D Lamb, M Gillooly, J Norman, D Morrison, C A D Smith, D J Harrison

Abstract

Aim—To investigate cytochrome P4501A1 (CYP1A1) polymorphism and susceptibility to emphysema and lung cancer.

Methods—A novel polymerase chain reaction (PCR) for genotyping the CYP1A1 polymorphism, corresponding to putative low or high enzyme activity, was developed to genotype lung cancer resection samples which had been assessed macroscopically for the presence of centriacinar and panacinar emphysema. Samples were collected and genotyped from a group of patients with chronic obstructive Airways disease. A control group of anonymous blood donations was genotyped to determine the basal levels of the polymorphism in the Scottish population.

Results—The high activity allele of the CYP1A1 gene is associated with susceptibility to centriacinar emphysema and lung cancer but not panacinar emphysema. CYP1A1 polymorphism is not linked to lung cancer in the absence of emphysema, nor to chronic obstructive Airways disease which is the clinical manifestation of emphysema, particularly of the panacinar type.

Conclusions—Susceptibility to emphysema and lung cancer is associated with polymorphism of the P4501A1 gene. A trend towards damage of centriacinar pattern has been detected, which supports the theory that centriacinar emphysema results from local, direct damage to the respiratory bronchioles from exposure to cigarette smoke.

Keywords: CYP1A1 polymorphism, emphysema, lung cancer, cigarette smoke.

A single inhalation of cigarette smoke contains approximately 10⁶ reactive species,¹ which are both cytotoxic and genotoxic. Lung tissue damage occurs through reactive species' destruction of cell membranes and structural components.² Procarcinogens and oxidants cause DNA mutations,³ which may lead to cancer initiation.⁴ Cigarette smoke can stimulate macrophages to release chemotactic factors which recruit inflammatory cells⁵ and nicotine has been shown to be chemotactic.⁶ These cells release proteases, particularly elastase, during their inflammatory response.⁷ In rats emphysema has been induced by administration of elastase to the lungs.⁸ Antiproteases protect the structure of the lung from destruction by proteases such as elastase and collagentase.⁹ Oxidants present in cigarette smoke inhibit the action of antiproteases, further reducing the protective capacity of the lungs.¹⁰ Cigarette smoke causes at least two diseases of major clinical importance, lung cancer and emphysema.

The lungs are protected from the toxic effects of cigarette smoke by antiproteases, antioxidant and xenobiotic metabolising enzymes.¹¹ In the lung both cancer and emphysema induced by cigarette smoke may result from variation in the protective capacity of lung tissue. Xenobiotic metabolising enzymes metabolise exogenous compounds, which may be toxic, to forms which are more easily excreted in the urine or bile.¹² This metabolism may be an important primary defense against lung injury resulting from exposure to cigarette smoke. Variation in interindividual expression of metabolising enzymes may result in the differential ability of tissues to protect against disease.

Several of the major xenobiotic metabolising enzymes are polymorphic at the genetic level. These include cytochrome P4502D6,¹³ glutathione S-transferase M1¹⁴ and N-acetyltransferase.¹⁵ Association between some of these enzyme polymorphisms and cancer susceptibility has been demonstrated¹⁶—for example, both cytochrome P4502D6 and glutathione S-transferase M1 polymorphisms have been associated with susceptibility to lung cancer, and the N-acetyl transferase 2 gene polymorphism has been implicated in bladder and colon cancers.

Cytochrome P4501A1 is a phase I metabolising enzyme which may activate procarcinogens and xenobiotics to their full carcinogenic and electrophilic forms.¹⁶ Expression of this enzyme is primarily extrahepatic¹⁷ and is widespread in the lung.¹⁸ CYP1A1 expression is inducible by polycyclic aromatic hydrocarbons such as benzo[a]pyrene and 3-methylcholanthrene,¹⁹ twenty which form a major component of cigarette smoke.²¹ A point mutation in exon 7 of the CYP1A1 gene results in an amino acid substitution from an isoleucine to a valine.²² This mutation occurs in the region of the gene which encodes the heme binding motif of the protein, and studies of benzo[a]pyrene metabolism have shown that the valine protein demonstrates almost twice the enzyme activity of the isoleucine protein.¹⁶ This polymorphism is linked to a MspI restriction enzyme fragment length polymorphism in the 3' region of the gene,²² which is associated
Figure 1 Schematic diagram of the CYP1A1 gene. Sequences of the two alleles of the CYP1A1 gene are shown, along with the downstream primer, which introduces a base change to the ampler. Following PCR, the base mismatch introduced into the ampler creates a NcoI site (box) in the Isoleucine allele only, the site is absent in the Valine allele.

with susceptibility to lung cancer in studies of Japanese populations. Studies of white populations have been unable to demonstrate similar associations, which may be a reflection of the lower prevalence of these polymorphisms in whites. Much larger study groups are required to investigate the polymorphism in the latter to generate sufficient numbers of rare alleles to permit statistical analysis of disease association.

To investigate involvement of the cytochrome P4501A1 gene polymorphism in susceptibility to lung disease, we collected lung samples which were resected for carcinoma. We also obtained blood samples from patients with chronic obstructive airways disease. Chronic obstructive airways disease is believed to be the clinical manifestation of emphysema; however, this clinical manifestation is more commonly seen in panacinar rather than centriacinar emphysema. Previous strategies for analysis of the exon 7 CYP1A1 polymorphism have made use of specific differential polymerase chain reaction (PCR) priming of the allelic variants of the gene, and have relied on high specificity of priming. We developed a novel PCR assay to genotype paraffin wax embedded lung tissues for the exon 7 polymorphism of the CYP1A1 gene, as use of high specificity primers relies on very stringent conditions which may reduce the yield of PCR amplimers.

Methods

Lung resection specimens (n=129) from smokers with lung cancer were collected. The presence, type and extent of emphysema was assessed macroscopically in non-involved lobes and this assessment was confirmed by morphometric microscopic analysis of alveolar wall surface area per unit volume (AWUV). All emphysema cases were of mild or moderate disease severity, with a forced expiratory volume in one second (FEV1) of at least 1-4, as patients must be sufficiently fit to recover from surgery.

Blood samples collected from a blood donor clinic served as an anonymous control popula-

DNA extraction and PCR analysis

DNA extraction from paraffin wax embedded lung samples with no evidence of carcinoma and blood samples was carried out as described previously. Genotyping was carried out by PCR analysis using buffer and 1-5 mM MgCl2 (Promega, Southampton, UK), 150 mM deoxyribonucleotides (Pharmacia, Milton Keynes, UK), 5% DMSO (Sigma, St Louis, Missouri, USA), 25 pmoles primer (Oswell DNA Services, Edinburgh, UK), and 2-5 units of Taq polymerase (Promega, UK). The primers used were: upstream, 5'-AAAGGCTGGG-TCCACCCCTT-3'; and downstream, 5'-AAAGGCTGTCGCCGAGCGCA-3' (fig 1).

The downstream primer incorporated a mismatched base to engineer a NcoI restriction enzyme site in the PCR products derived from the Ile462 allele of the gene. This restriction site is lost in the Val462 allele of the gene. The primers amplify both alleles and the genotypes are distinguished by NcoI digestion of the products. A NcoI restriction enzyme site located upstream of the mutation in either genotype serves as a positive control for PCR product digestion. PCR products were electrophoresed in 3% NuSieve and SeaKem agarose (FMC Bioproducts, Rockland, Maine, USA), and restriction enzyme digestion fragments were electrophoresed in 3% Metaphor agarose (FMC Bioproducts).

Statistical analyses

Odds ratios and confidence intervals were used to analyse the frequencies of the CYP1A1 genotypes, significance testing was by χ² analysis.

Results

Disease information

No significant differences in age, sex, or tumour type were found within the study populations. The median FEV1 of chronic obstructive airways disease cases was 1-1, and 2-2 for the biopsy study group. Smoking histories were recorded where possible in pack years of exposure, and the median pack years for the chronic obstructive airways disease group was 35, while the biopsy cases had a median of 46 pack years.

Of the 129 lung cancer samples studied, 42 had no macroscopic emphysema, 34 showed centriacinar patterns of damage, 17 samples had panacinar emphysema, and 36 lung biopsy specimens had both centriacinar and panacinar forms of emphysema.

Genotyping of CYP1A1 gene polymorphism

Each ampm of analysed had a diagnostic NcoI restriction enzyme site and a second constant control NcoI site, which enabled distinction of the CYP1A1 genotype of the individual. PCR analysis and subsequent enzyme restriction
Table 1 CYP1A1 genotypes for the disease groups and control group for statistical comparison

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Ile/Ile genotype</th>
<th>Ile/Val genotype</th>
<th>Val/Val genotype</th>
<th>Total cases</th>
<th>p value</th>
<th>Odds ratio</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>245 (87%)</td>
<td>33 (12%)</td>
<td>3 (1%)</td>
<td>281</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>All lung cancers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(82%)</td>
<td>106 (21%)</td>
<td>21 (2%)</td>
<td>2 (19%)</td>
<td>129</td>
<td>1:81</td>
<td>1:48</td>
<td>0:83-2:61</td>
</tr>
<tr>
<td>Cancer with no emphysema</td>
<td>30 (93%)</td>
<td>3 (7%)</td>
<td>0 (0%)</td>
<td>42</td>
<td>1:11</td>
<td>0:52</td>
<td>0:16-1:77</td>
</tr>
<tr>
<td>Cancer with emphysema</td>
<td>47 (77%)</td>
<td>18 (21%)</td>
<td>2 (2%)</td>
<td>87</td>
<td>5:33</td>
<td>2:03</td>
<td>1:10-5:75</td>
</tr>
<tr>
<td>Chronic obstructive airways disease</td>
<td>42 (82%)</td>
<td>9 (18%)</td>
<td>0 (0%)</td>
<td>51</td>
<td>1:29</td>
<td>1:46</td>
<td>0:66-3:25</td>
</tr>
</tbody>
</table>

Table 2 CYP1A1 genotypes of cases with emphysema and lung cancer divided by pattern of emphysema

<table>
<thead>
<tr>
<th>Types of emphysema</th>
<th>Ile/Ile genotype</th>
<th>Ile/Val genotype</th>
<th>Val/Val genotype</th>
<th>Total cases</th>
<th>p value</th>
<th>Odds ratio</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centriacinar alone</td>
<td>23 (73-5%)</td>
<td>9 (23-5%)</td>
<td>0 (0%)</td>
<td>34</td>
<td>4:62</td>
<td>0:035</td>
<td>1:06-5:67</td>
</tr>
<tr>
<td>Panacinar alone</td>
<td>15 (88%)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>17</td>
<td>0:02</td>
<td>0:91</td>
<td>0:20-4:10</td>
</tr>
<tr>
<td>Both</td>
<td>27 (75%)</td>
<td>8 (22%)</td>
<td>1 (3%)</td>
<td>36</td>
<td>3:89</td>
<td>2:25</td>
<td>0:98-5:17</td>
</tr>
</tbody>
</table>

Figure 2 (A) Results of a PCR of the CYP1A1 gene. DNA molecular marker V is shown in the left hand lane, with PCR amplimers in the remaining lanes. The amplimer (322 base pairs) lies between fragments of 434 and 267 base pairs of the marker. (B) Results of a NcoI digestion of the CYP1A1 amplimer. The two bands represent the two alleles of the gene, the higher molecular weight fragment (250 base pairs) representing the uncut valine allele of the gene, and the lower molecular weight band (231 base pairs) representing the cut isoleucine allele of CYP1A1. Lanes 1, 5 and 6 therefore represent heterozygotes for the two alleles, while lanes 2, 3 and 4 contain digests from individuals homozygous for the common isoleucine allele.

Discussion

We have found a statistically significant association of the rare, putative high activity allele at the Ile<sup>462</sup> allele, 16% (21/129) were heterozygous for Ile<sup>462</sup> while 2% (three of 129) exhibited Val<sup>462</sup> homozygosity. This group was not significantly different from the control population.

In the group of patients with chronic obstructive airways disease, of the 51 samples, 82% (42/51) were homozygous for Ile<sup>462</sup> and 18% (nine of 51) were heterozygous for the polymorphism. No individuals homozygous for the valine allele were found in this disease group, and no significant difference between this study group and the controls was seen.

To investigate the CYP1A1 gene polymorphism and susceptibility to emphysema, the total lung cancer study group was divided according to the pattern of emphysematous damage in the lung (table 2). When this was done, 42 samples showed no evidence of emphysema and of these, 93% (39/42) were Ile<sup>462</sup> homozygotes; 7% (three of 42) were heterozygotes. Centriacinar emphysema only was found in 34 cases and of these, 73-5% (25/34) were homozygous for the valine allele and 26-5% (nine of 34) were Ile/Val. Panacinar emphysema alone was present in 17 samples and 88% (15/17) were Ile<sup>462</sup> homozygotes, 6% (one of 17) were Ile/Val, and 6% (one of 17) were homozygous for the Val<sup>462</sup> allele. Both panacinar and centriacinar emphysema were found in 36 of the lung cancer samples and homozygotes for the isoleucine allele accounted for 75% (27/36) of these cases, 22% (eight of 36) were heterozygotes and 3% (one of 36) were Val<sup>462</sup> homozygotes.

Of these disease groups, only those patients with centriacinar emphysema alone and both centriacinar and panacinar emphysema differed significantly from the controls. Odds ratios were 2-45 (95% confidence limits 1-06-5-67) and 2-25 (95% confidence limits 0-98-5-17), respectively.

Discussion

We have found a statistically significant association of the rare, putative high activity allele.
of CYP1A1 in cases with both lung cancer and emphysema (odds ratios of 2.45 and 2.25) but not in cases with chronic obstructive airways disease or cancer alone. Clinical cases of chronic obstructive airways disease do not represent just emphysema but also include chronic obstructive airways disease secondary to chronic bronchitis. This may explain why a lack of CYP1A1 association is seen for this condition, but association with the polymorphism is seen with pathological emphysema. Previous studies investigating CYP1A1 and lung cancer have provided conflicting data. Whereas in Japanese populations CYP1A1 conferred a threefold increased risk of lung cancer, similar studies of Scandinavian populations have failed to find any association. No study to date has considered the possibility that CYP1A1 may confer susceptibility not just to lung cancer but also to other forms of lung disease, such as emphysema. What do the results we have obtained suggest?

Firstly, although our data indicate that CYP1A1 is associated with lung cancer and emphysema, the increased relative risk we have found is rather small and is only significant when both diseases are present. However, emphysema progressing to lung cancer are extremely prevalent diseases, hence even a slight increase in disease susceptibility conferred by this polymorphism may account for a large proportion of cases.

Secondly, the increased susceptibility to lung disease conferred by the CYP1A1 polymorphism appears to be an early event, occurring at a stage before the injury response pathway leading to cell death and inflammation has diverged from that leading to mutagenesis and eventual tumour progression. This is in keeping with the likely position of CYP1A1 in the pathway of cigarette smoke metabolism—that is, it is proximate to the insult by nature of its expression in lung parenchyma and role in phase I metabolism.

Thirdly, the effect of CYP1A1 genotype and phenotype on disease susceptibility will be influenced by other mechanisms of disease development. These may be more important than CYP1A1 in determining the likelihood of cigarette smoke injury to result in predominantly genotoxic or cytotoxic injury. Thus, certain protective mechanisms such as the glutathione dependent system, epoxide hydrolase, differences in proteolytic enzymes, or anti-proteases may reduce the likelihood of cell damage caused by lipid or protein peroxidation leading to emphysema. It is perhaps significant that the cases with both lung cancer and emphysema had mild or moderate emphysema whereas the clinical group with chronic obstructive airways disease had severe disease—that is, CYP1A1 polymorphism contributes little towards susceptibility to severe, non-occupational lung injury.

Conversely, differences in DNA repair and other genes involved in recognising and eliminating DNA damage may be very important in determining whether or not tumorigenesis can occur. These mechanisms are likely to differ between individuals and consistent with this is the presence of known polymorphisms/mutations—for example, in the p53 gene and in the DNA mismatch repair system. These polymorphisms or mutations may contribute to interindividual differences in the capacity to eliminate DNA damage and therefore prevent mutations leading to tumorigenesis.

Only in cases with no bias towards, or away from, non-neoplastic or neoplastic lung disease would CYP1A1 be expected to have a detectable effect on the susceptibility to disease rather than simply a more subtle underlying modification of mechanisms and responses to injury.

The results that we have obtained in this study show that a genetically determined difference in xenobiotic metabolism, in this case components of cigarette smoke, may alter the response of cells or tissue to injury and thus susceptibility to disease.

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