CYP1B1 Polymorphisms and K-ras Mutations in Patients with Pancreatic Ductal Adenocarcinoma

Marta Crous-Bou · Immaculata De Vivo · Miquel Porta · José A. Pumarega · Tomás López · Joan Alguacil · Eva Morales · Núria Malats · Juli Rifa · David J. Hunter · Francisco X. Real · For the PANKRAS II Study Group

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Abstract The frequency of CYP1B1 polymorphisms in pancreatic cancer has never been reported. There is also no evidence on the relationship between CYP1B1 variants and mutations in ras genes (K-, H- or N-ras) in any human neoplasm. We analyzed the following CYP1B1 polymorphisms in 129 incident cases of pancreatic ductal adenocarcinoma (PDA): the m1 allele (Val to Leu at codon 432) and the m2 allele (Asn to Ser at codon 453). The calculated frequencies for the m1 Val and m2 Asn alleles were 0.45 and 0.68, respectively. CYP1B1 genotypes were out of Hardy–Weinberg equilibrium; this was largely due to K-ras mutated PDA cases. The Val/Val genotype was over five times more frequent in PDA cases with a K-ras mutation than in wild-type cases (OR = 5.25; P = 0.121). In PDA, polymorphisms in CYP1B1 might be related with K-ras activation pathways.

Keywords Pancreatic neoplasms · CYP1B1 polymorphisms · K-ras oncogene · ras genes

Abbreviations PANKRAS II study group—Members of the multicenter prospective study on the role of K-ras and other genetic alterations in the diagnosis, prognosis and etiology of pancreatic and biliary diseases (PANKRAS II) study group are mentioned in previous publications.

M. Crous-Bou · M. Porta (✉) · J. A. Pumarega · T. López · J. Alguacil · E. Morales · N. Malats · F. X. Real
Institut Municipal d’Investigació Médica (IMIM), CIBER de Epidemiología y Salud Pública (CIBERESP), Carrer del Dr. Aiguader 88, 08003 Barcelona, Spain
E-mail: mporta@imim.es

M. Crous-Bou · M. Porta
School of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain
I. De Vivo · D. J. Hunter
Harvard School of Public Health, Boston, MA, USA

I. De Vivo · D. J. Hunter
Channing Laboratory, Brigham and Women’s Hospital, Boston, MA, USA

J. Alguacil
Department of Environmental Biology & Public Health, Universidad de Huelva, Huelva, Spain

J. Rifa
Hospital Son Dureta, Palma de Mallorca, Spain

F. X. Real
Universitat Pompeu Fabra, Barcelona, Spain

Introduction

The cytochrome P450 gene superfamily participates in the oxidative metabolism of drugs and endogenous substrates, including steroids, and in the metabolic activation of exogenous chemical carcinogens such as polycyclic aromatic hydrocarbons (PAH), nitroaromatics, and arylamines [1–5]. Cytochrome P4501B1 (CYP1B1) is a carcinogen- and estrogen-metabolizing enzyme with an important role in the bioactivation of some environmental procarcinogens.
CYP1B1 activates PAH, including benzo[a]pyrene and 7,12-dimethylbenzo[a]anthracene (DMBA), as well as heterocyclic aromatic amines derived from tobacco smoke and some occupational exposures, which are carcinogens in experimental animals [1, 2]. Moreover, the oxidant steps catalyzed by this enzyme often create more reactive intermediates able to bind with DNA, leading to DNA adduct formation and genetic mutations [8].

CYP1B1 polymorphisms are hypothesized to influence interindividual differences in susceptibility to chemically induced cancers [3, 9, 10]. Two common functional polymorphisms in CYP1B1 have been described: V432L (Val to Leu at codon 432, the m1 allele) and A453S (Asn to Ser at codon 453, the m2 allele) [9, 11]. CYP1B1 polymorphisms have been studied in human cancer, including breast, ovarian, endometrial, lung, and bladder cancers, but their distribution in pancreatic cancer is unknown [1, 3, 6–8, 10, 12–15]. There is also no evidence on the potential relationship between CYP1B1 variants and K-ras mutations, the oncogene most frequently mutated in pancreatic ductal adenocarcinoma (PDA).

Exposure to PAH and aromatic amines, mainly through smoking and occupation, has been related with pancreatic carcinogenesis [16–19]. Some studies suggest that lifestyle and environmental factors may influence the occurrence or persistence of K-ras mutations [20–25]. Thus, K-ras mutated and K-ras wild-type PDAs may develop through different pathways, probably involving different gene–environment interactions; however, findings remain inconclusive [22–24, 26].

The main objective of the present study was to analyze the distribution of polymorphisms in the m1 and m2 alleles of CYP1B1 among patients with pancreatic ductal adenocarcinoma. We also analyzed such polymorphisms by K-ras mutational status.

**Patients and Methods**

**Selection of Patients and Interviews**

Methods have been previously described in detail [22–24, 26, 27]. Briefly, subject recruitment took place at five general hospitals in the eastern Mediterranean part of Spain, where 185 incident cases of PDA were prospectively identified. Twenty-nine controls were recruited at one of the hospitals; they were admitted for benign, non-digestive disorders unrelated to tobacco and alcohol consumption [24]. The present report is based on 129 PDA patients with m1 and m2 CYP1B1 polymorphisms analyzed and on 87 cases who also had information about K-ras status. There were no significant differences between them and the remaining cases according to education, social class, sex, occupation, hospital, tumor stage, signs and symptoms, duration of the interview, caloric intake, and consumption of coffee, tobacco, and alcohol, except for cases included for K-ras analysis who were slightly younger [24, 26]. The ethics committees of the participating hospitals approved the study protocol, and patients gave informed consent to be included in the study. A structured form was used to collect clinicopathological information from medical records. Over 88% of the patients were interviewed face-to-face by trained monitors during hospital stay, close to the time of diagnosis. Interviews included questions about past clinical history, occupation, diet, and coffee, alcohol, and tobacco consumption [22–24, 26, 27].

**Identification of CYP1B1 Polymorphisms**

Details of laboratory protocols have been described elsewhere [12, 13]. DNA was extracted from buffy coat fractions using the Qiagen QIAamp blood kit (Qiagen, Inc., Chads-worth CA). Genotyping was performed by automated DNA sequencing on the ABI 377X using BigDyeterminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA). Two common functional polymorphisms in CYP1B1 were examined: V432L and A453S. Because of the close proximity of m1 to m2, within 60 bp, we were able to amplify both polymorphisms within one amplicon. Polymerase chain reaction (PCR) amplification of m1 and m2 was generated using the primers 5′-CCAACACCTCTGTTTGGGA-3′ and 5′-GCTCATTTGGTGCCCTG-3′. Heterozygotes were denoted at positions where the secondary peak height was ≥45–50% of the primary peak height in both forward and reverse sequence reads for a subset of the samples. After establishing the criteria for base calling, the forward PCR primer was used for all subsequent sequencing reactions.

**Detection of K-ras Mutations**

Details of laboratory protocols have been described elsewhere [22–24, 26]. Briefly, mutations in codon 12 of K-ras oncogene were studied using DNA extracted from paraffin-embedded tumor tissue. Amplifications were done in two steps by nested PCR; an artificial BstNI restriction endonuclease site was introduced to discriminate between wild-type and mutated K-ras codon 12 sequences. Products were analyzed by acrylamide gel electrophoresis and ethidium bromide staining. This technique was able to detect one homozygous mutated cell in the presence of 102 normal cells. To characterize the nucleotide substitution in codon 12, all mutated samples were further analyzed using a similar restriction fragment-length polymorphism (RFLP)-based...
approach. Interpretation of digestion products’ electrophoresis was performed independently by two investigators to confirm the results.

Data Analysis

Allele frequencies and Hardy–Weinberg equilibrium were calculated for the m1 and m2 polymorphisms in the 129 patients with PDA and in the 19 hospital controls. We also compared the m1 and m2 genotypes of the 65 cases of PDA with a K-ras mutated tumor and the 22 cases of PDA whose tumors did not harbor such mutations. In the analyses of CYP1B1 by K-ras status, Val/Leu and Leu/Leu were combined in one group, and Asn/Ser and Ser/Ser were also combined. Univariate statistics were computed as customary [28, 29]. In contingency tables, Fisher’s exact test was applied to assess the relationship between two categorical variables. To estimate the magnitude of the associations, multivariate-adjusted odds ratios and their corresponding 95% confidence intervals (CI) were calculated by unconditional logistic regression. These analyses were performed using SPSS, version 12.0 (SPSS Inc, Chicago, IL) [28, 29]. Age, sex, tobacco, and alcohol and coffee consumption were assessed in the models as potential confounders. The level of statistical significance was set at 0.05, and all tests were two-tailed.

Results

In the 129 patients with PDA the frequencies for m1 Val and Leu alleles were 0.45 and 0.55, respectively, and among controls they were 0.50 and 0.50, respectively. For m2 Asn and Ser alleles the frequencies were 0.68 and 0.32 in cases and 0.73 and 0.27 in controls, respectively (Table 1).

Table 1 Genotype frequencies of the m1 and m2 alleles among cases of pancreatic ductal adenocarcinoma with and without mutations in codon 12 of the K-ras oncogene, and among hospital controls

<table>
<thead>
<tr>
<th>CYP1B1</th>
<th>All PDA cases</th>
<th>K-ras mutated PDA cases</th>
<th>K-ras wild-type PDA cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1 (codon 432)</td>
<td>N = 129</td>
<td>N = 65</td>
<td>N = 22</td>
<td>N = 19</td>
</tr>
<tr>
<td>Val/Val</td>
<td>19 (14.7)</td>
<td>13 (20.0)</td>
<td>1 (4.6)</td>
<td>4 (21.1)</td>
</tr>
<tr>
<td>Val/Leu</td>
<td>79 (61.3)</td>
<td>40 (61.5)</td>
<td>16 (72.7)</td>
<td>11 (57.8)</td>
</tr>
<tr>
<td>Leu/Leu</td>
<td>31 (24.0)</td>
<td>12 (18.5)</td>
<td>5 (22.7)</td>
<td>4 (21.1)</td>
</tr>
<tr>
<td>m2 (codon 453)</td>
<td>N = 126</td>
<td>N = 63</td>
<td>N = 21</td>
<td>N = 18</td>
</tr>
<tr>
<td>Asn/Asn</td>
<td>54 (42.9)</td>
<td>25 (39.7)</td>
<td>9 (42.9)</td>
<td>9 (50.0)</td>
</tr>
<tr>
<td>Asn/Ser</td>
<td>64 (50.8)</td>
<td>33 (52.4)</td>
<td>11 (52.4)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>8 (6.3)</td>
<td>5 (7.9)</td>
<td>1 (4.7)</td>
<td>1 (5.6)</td>
</tr>
</tbody>
</table>

Note: Values in parentheses are column percentages

Genotype frequencies were in Hardy-Weinberg equilibrium among controls (P = 0.500 and 0.722 for m1 and m2, respectively). They were out of Hardy–Weinberg equilibrium in PDA cases: clearly so for m1 (P = 0.007) and probably so for m2 (P = 0.053).

In the m1 allele, the Val/Val genotype was slightly less frequent in PDA cases than in controls (14.7% versus 21.1%); accordingly, heterozygotes, and homozygotes for Leu were more frequent in cases than controls. For the m2 allele, the proportion of Asn homozygotes was slightly lower in PDA cases than in controls (42.9 versus 50.0), while Ser genotypes were slightly more frequent in PDA cases than in controls (Table 1).

K-ras mutated cases were more likely to be homozygous for Val/Val in m1 than K-ras wild-type cases (crude OR = 5.25; P = 0.121). When age, sex, tobacco, and coffee and alcohol consumption were adjusted for, the corresponding OR was 6.41 (P = 0.111) (Table 2).

Table 2 Relationship between m1 and m2 polymorphisms in CYP1B1 and the frequency of K-ras mutations in patients with pancreatic ductal adenocarcinoma

<table>
<thead>
<tr>
<th>K-ras</th>
<th>Mutated (N = 65)</th>
<th>Wild type (N = 22)</th>
<th>Unadjusted OR</th>
<th>Adjusted ORa</th>
<th>Adjusted ORb</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1 (codon 432)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Leu</td>
<td>52 (80.0)</td>
<td>21 (95.5)</td>
<td>1.00</td>
<td>0.121</td>
<td>1.00</td>
</tr>
<tr>
<td>Val/Val</td>
<td>13 (20.0)</td>
<td>1 (4.5)</td>
<td>5.25</td>
<td>(0.65–42.71)</td>
<td>6.41</td>
</tr>
<tr>
<td>m2 (codon 453)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn/Asn</td>
<td>25 (39.7)</td>
<td>9 (42.9)</td>
<td>1.00</td>
<td>0.797</td>
<td>1.00</td>
</tr>
<tr>
<td>Any Ser</td>
<td>38 (60.5)</td>
<td>12 (57.1)</td>
<td>1.14</td>
<td>(0.42–3.10)</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Note: values in parentheses are column percentages, except where otherwise stated

The first category of each variable is the reference category (odds ratio [OR] = 1.00). CI: confidence interval

a Odds ratio adjusted by age, sex, tobacco, and coffee and alcohol consumption

b P-value derived from Wald’s test
Genotype frequencies in the m2 allele did not differ among PDA patients with and without mutations in K-ras.

Discussion

We analyzed two CYP1B1 polymorphisms in 129 patients with PDA, Val432Leu and Asn453Ser, both in exon 3, a critical region for the catalytic function of the enzyme [9, 11, 15]. The observed frequencies of Val and Asn alleles were 0.45 and 0.68, respectively. The prevalence of the m1 Val allele was similar to that previously described in Caucasian populations; the prevalence of the m2 Asn allele was lower than in other reports [12, 13]. Different allele frequencies have been observed in Caucasian, African, and Asian populations [6, 10–15].

The absence of Hardy–Weinberg equilibrium of the m1 polymorphism might reflect that CYP1B1 is involved in the pathogenesis of pancreatic cancer. Unfortunately, CYP1B1 polymorphisms have not been studied before in pancreatic cancer. Furthermore, to our knowledge no reports are available on the relationship between CYP1B1 variants and mutations in ras genes (K-, H- or N-ras) in any human neoplasm.

Our results raise the possibility that, as compared to patients with any Leu genotype in m1, individuals with the Val/Val genotype might have a higher probability of a K-ras mutated PDA than of a K-ras wild-type tumor. However, our sample size was low for the case–case and case–control comparisons and hence independent studies are required to refute or replicate the findings. CYP1B1 is related to PAHs metabolism and smoking is a well-established risk factor for PDA [8, 18, 20]. The Val/Val genotype might be associated with poor detoxification activity and, consequently, with an impaired elimination of some environmental factors such as tobacco smoke or occupational exposures. Some of these compounds may be oxidized to electrophilic metabolites that can react with DNA to form stable adducts, which may favor mutations in K-ras and other oncogenes [1, 2, 8, 30].

In conclusion, our results provide the first analysis of CYP1B1 variants in pancreatic cancer, and suggest a possible relationship between the m1 locus of CYP1B1 and K-ras mutations in patients with PDA. Further studies are warranted to elucidate the role of CYP1B1 variants in pancreatic cancer and their relation with K-ras activation pathways.

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