SHORT COMMUNICATION

Influence of a mutation reducing the catalytic activity of the cytochrome P450 CYP2D6 on lung cancer susceptibility

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The possible association between lung cancer and the CYP2D6*9 mutant allele, which reduces the catalytic activity of cytochrome P450 CYP2D6, was examined by PCR-SSCP using peripheral blood DNA from 249 cases of lung cancer and 265 controls, with detailed data on smoking. The CYP2D6*9 mutant allele was present in 4.9% of controls and 6% of cases. Adjusted for age, hospital and smoking, the odds ratio (OR) of lung cancer associated with the presence of the CYP2D6*9 mutant allele was 1.2 [95% confidence interval (CI) 0.5-2.9]. According to histological type, adenocarcinoma and small cell carcinoma were not associated with the presence of the CYP2D6*9 mutant allele and a non-significant higher occurrence of the mutant allele was observed for squamous cell carcinoma (OR 1.74, 95% CI 0.6-4.8). Moreover, no associations were observed upon stratification by number of pack-years of cigarette smoking. These results do not confirm an earlier report that this CYP2D6*9 mutant allele may be an additional risk factor for the development of lung cancer.

CYP2D6 is part of a gene cluster on chromosome 22 which encodes for a polymorphic microsomal cytochrome P450 isomerase involved in the metabolism of numerous drugs, environmental toxic chemicals and probably endogenous substances (1-7). Genetic polymorphism of the cytochrome P450 CYP2D6 debrisoquine hydroxylase gene locus is an autosomal recessive trait dividing the Caucasian population into three phenotypes: extensive metabolizers (EM*), intermediate metabolizers (IM) and poor metabolizers (PM) (8,9). Inter-individual differences in the metabolic capacity of CYP2D6 may be expected to be a key factor in susceptibility to develop cancers where environmental chemicals are implicated (10,11). The inter-individual variations in the activity of CYP2D6 are closely linked to mutant alleles encoding CYP2D6 (12). Identification of mutations responsible for CYP2D6 polymorphism has allowed the development of genotyping assays using PCR to detect each of them in genomic DNA samples and thereby to predict the alteration of metabolic capacities for CYP2D6 substrates (13-17). Using these assays, many authors have extensively studied the distribution of CYP2D6 alleles in control populations and different cancer groups (18-21).

Recently, one of these studies suggested that the CYP2D6*9 mutant allele was 6-fold more frequent among patients with lung cancer than in controls (22). The CYP2D6*9 mutant allele is characterized by a single 3 bp deletion in exon 5 of the CYP2D6 gene (mutation D6-C) and is carried by a 29 kb fragment (haplotype 29-C) (17,23). Individuals who carry this mutation have an altered ability to metabolize CYP2D6 substrates and are IM (23). In order to investigate the hypothesis of an association between the mutation D6-C and lung cancer susceptibility, we have used a procedure recently developed to analyse the gene sequence variations based on SSCP analysis of CYP2D6 fragments amplified by PCR (PCR-SSCP) (24). This method of detection of the mutation D6-C has been used in a case-control study including 249 patients with lung cancer and 265 controls males matched for age, hospital and residence area.

We analysed DNA extracted from peripheral blood samples of French patients in a lung cancer case-control study. A detailed description of the methods of subject enrolment and study population has been recently published (25). In brief, three French hospitals recruited all patients. They were Caucasian males <75 years old with a histologically confirmed lung cancer diagnosis and no previous cancer diagnosis. Male controls were matched for age, hospital and residence area. No controls with non-cancerous lung diseases or any kind of cancer were included. Questionnaire data on smoking habits were collected and, as expected, smoking was much more common for patients with cancer than controls. DNA samples from 514 subjects were subjected to PCR-SSCP analysis of exon 5 of CYP2D6 according to a procedure previously described (24). Briefly, a large fragment encompassing all exons and introns of the gene was first amplified from genomic DNA using primers specific for the 5' and 3' flanking regions of the gene. This fragment was then used as a template to amplify exon 5 of the gene and its 3' and 5' proximal flanking regions with a pair of nested primers. SSCP profiles obtained for exon 5 of the CYP2D6 gene were compared with those from reference DNA samples which corresponded to a normal sequence or a known mutated sequence of exon 5, previously characterized by sequencing (24). All statistical analysis was performed on a VAX (Digital Equipment). For the univariate analysis and the adjustment for single confounders we used Statistical Analysis Software (SAS). Odds ratios (OR) as estimators of relative risk were calculated with their 95% confidence intervals (CI).

Comparison of SSCP profiles obtained from the tested individuals with reference DNA samples allowed the identification of individuals with the CYP2D6*9 mutant allele or the CYP2D6*1A wild-type allele (mutation D6-C absent). As previously described, each subject heterozygous for deletion (D6-D) of the entire CYP2D6 gene (D6-C/D6-D or D6-WT/D6-D) appeared homozygous (D6-C/D6-C or D6-WT/D6-WT) in SSCP patterns (24). Consequently, to identify subjects with the mutation D6-D, we combined our PCR-SSCP data with those obtained from a Xbal RFLP analysis recently published.
mutation D6-C and susceptibility to lung cancer, we screened cancer risk according to the number of pack-years of cigarette smoking. No association was found among moderate (pack-years 0.6–4.8). We found no major association with the risk of lung cancer. The majority of cases and controls were identified in exon 5 of CYP2D6 gene and to simultaneously screen for new mutations (24). This method has already proved its efficacy for the characterization of previously known alleles of the CYP2D6 gene and for identification of new mutant alleles of the gene (26–28; Marez et al., in preparation). We thought that this approach would advantageously replace the currently available methods for CYP2D6 genotyping in order to analyse more accurately, simply and rapidly the apparent linkages between this gene and lung cancer.

Within the control population, the frequency of the mutant allele (0.030) was representative of a healthy European population and reflected an adequate study design (16). Moreover, our large case–control study allowed us to examine the hypothesis of a link between the mutation D6-C and lung cancer with sufficient statistical power. Clearly, the OR for the presence of the CYP2D6*9 mutant allele in relation to lung cancer risk did not support a major association between the presence of this mutant allele and lung cancer.

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presence of the variant allele and lung cancer risk according to pack-years of smoking. No association was found when moderate and heavy smokers were distinguished (Table II).

These findings appear at variance with those of Agundez et al., which showed that the CYP2D6*9 mutant allele was 6-fold more frequent in patients with lung cancer than in controls. In our large case-control study, subjects were matched for age, sex, hospital and residence area, whereas in the previous study there was no matching of variables and fewer subjects were studied. In contrast to the Spanish study, we have estimated the adjusted OR using an unconditional logistical regression model that includes two matching variables (age and hospital) and the exposure variable (e.g. tobacco exposure) as well as the CYP2D6 gene. Agundez et al. provided no information on the mean number of pack-years of cigarettes consumed by controls and, consequently, they did not include tobacco exposure in their statistical analysis (22). Finally, variations in environmental background of the populations studied may be an additional factor in the discrepancy. These differences between the two populations and methods might explain the discrepant findings.

In conclusion, the results of this study do not support evidence for a direct link between the CYP2D6*9 mutant allele and susceptibility to lung cancer. However, it is possible that other mutations reducing the catalytic activity of CYP2D6 or causing changes in the CYP2D6 substrate specificity might be involved, alone or in combination, in lung cancer development. Using our large case-control population, rapid PCR-SSCP analysis of all exons of the CYP2D6 gene. Agundez et al. provided no information on the mean number of pack-years of cigarettes consumed by controls and, consequently, they did not include tobacco exposure in their statistical analysis (22). Finally, variations in environmental background of the populations studied may be an additional factor in the discrepancy. These differences between the two populations and methods might explain the discrepant findings.

Acknowledgement

This study was supported by the Centre Hospitalier Régional et Universitaire de Lille.

References

Received on April 17, 1996; revised on June 14, 1996; accepted on June 21, 1996.