Polymorphism in CYP1A1 and CYP2D6 Genes: Possible Association with Susceptibility to Lung Cancer

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Polymorphism of CYP2D6 gene encoding for debrisoquine hydroxylase was determined genotypically for 94 controls and 77 lung cancer patients using a polymerase chain reaction-based application. Both of the point mutations that give rise to deficient alleles of the CYP2D6 gene are detectable by this method. Out of the 94 healthy controls, 3 individuals (3.2%) had poor metabolizer (PM) genotypes, whereas no PM genotypes were detected in the lung cancer patient group. We observed no difference in the allelic frequencies for either homozygous extensive metabolizers (EMs) or heterozygous EMs between the lung cancer patients and the healthy controls. However, the absence of the poor metabolizer genotype (0/77) in the lung cancer patients is compatible with the hypothesis that there is an increased risk of lung cancer for individuals who are extensive metabolizers of debrisoquine. Another member of the cytochrome P450 gene superfamily that has attracted interest for its potential role in human pulmonary carcinogenesis is the CYP1A1 gene. In CYP1A1 gene studies, a polymorphic site assessable to MspI gives rise to two different hybridizable fragments in a Southern blot analysis (alleles C1 and C2, respectively). The C2C2 genotype has previously been associated with an increased risk of lung cancer. So far 74 lung cancer patients, 30 patients with lung diseases other than cancer, and 118 healthy controls have been studied for CYP1A1 gene polymorphism. No association between the MspI restriction fragment length polymorphism in the CYP1A1 gene and lung cancer susceptibility has been found.

Introduction

Individual differences in the ability to metabolize xenobiotics may be a key factor in the genetic predisposition, or host susceptibility, to various carcinogens (1,2). The cytochrome P450-dependent mono-oxygenases are important in the metabolism of environmental carcinogens. Among the most studied genetic variables concerning P450 enzymes are the polymorphisms of the genes CYP2D6 and CYP1A1, which encode for debrisoquine 4-hydroxylase and aryl hydrocarbon hydroxylase, respectively (3). Both polymorphisms have recently been associated with susceptibility to lung cancer (1,2).

The debrisoquine metabolism phenotype has been determined conventionally by administering the test drug and subsequently determining the urinary metabolic ratio (4). However, this phenotyping procedure has some limitations because of confounding and the potential adverse effects of the drugs (5). Extensive metabolizers (EMs) can either be homozygous or heterozygous for the normal allele, the metabolic ratio being lower in the latter, but the two genotypes cannot be reliably distinguished by phenotyping (6).

Recently, a functional CYP2D6 allele and two related genes, designated CYP2D7 and CYP2D8P (pseudogene), have been isolated and completely sequenced (7). This has allowed CYP2D6 genotyping using polymerase chain reaction (PCR)-based methods (8,9). These methods do not have the same limitations as the conventional phenotyping analyses and they can be accomplished using only tiny amounts of white blood cell DNA.

Another P450 enzyme that is of considerable interest for its potential role in human pulmonary carcinogenesis is aryl hydrocarbon hydroxylase (AHH). AHH activity can be induced by polycyclic aromatic hydrocarbon (PAH) components of cigarette smoke condensate (10,11). A significant correlation between high AHH-inducibility and enhanced risk of bronchogenic carcinoma in cigarette smokers was originally reported by Kellerman et al. (12), and subsequent studies have led to the conclusion that the cigarette smokers with the high inducibility phenotype are several times more prone to bronchogenic carcinoma than smokers with the low inducibility phenotype (13). The
CYPIAl gene encoding for AHH thus attracts particular interest for its role in individual inducibility status. Restriction fragment length polymorphisms (RFLPs) are now commonly used for diagnosing and predicting a growing number of clinical disorders and for human family studies. Determination of the cDNA sequence of the CYPIAl gene (14) has made it possible to look for the RFLPs in the CYPIAl gene too. In the early attempts, no association between RFLP patterns and AHH inducibility was noted (14). The recent discovery of MspI polymorphism in the last intron of the CYPIAl gene (15), however, has led to the detection of cosegregation of the P450IA1 phenotype and MspI RFLP patterns (16). In addition, a strong correlation between enhanced lung cancer risk and MspI RFLP patterns of the CYPIAl gene was discovered in a Japanese study (17).

In this study, a combined application of RFLP-based methods (8,9) for determining debrisoquine hydroxylase polymorphism in leukocyte DNA was used to study the distribution of extensive (EM) and poor metabolizer (PM) genotypes in Finnish lung cancer patients and in control subjects. The method used was estimated to detect at least 95% of the known allelic variants of the PM genotype (18). The study populations were also screened for the MspI RFLP in the CYPIAl gene.

Subjects and Methods

Subjects

Three groups were examined in this study: the first group comprised 91 healthy blood donors at the Finnish Red Cross Blood Transfusion Service (all males) and 27 volunteers (12 males, 15 females) employed at the Institute of Occupational Health in Helsinki. The other two groups comprised 77 lung cancer patients (64 males, 13 females) and 30 patients with lung diseases other than cancer (21 males, 9 females), respectively.

The patients came to Helsinki University Central Hospital over a 3-year period for surgical pulmectomy or lobectomy because they had suspected, operable lung carcinoma. The mean age for the lung cancer patients was 62 years (SD 9.7), and most of them were either current cigarette smokers or ex-smokers for more than 3 years before surgery. According to interview data, only seven patients had never smoked. The blood samples were obtained the day before surgery. None of the patients had received chemotherapy or radiotherapy. The study design was approved by the local ethics committees.

DNA Analyses

DNAs were isolated from blood samples (10–20 mL) obtained from all the subjects. The DNA samples were analyzed by a PCR-genotyping assay and by a MspI RFLP analysis.

The PCR-based CYPI2D6 genotyping analyses were performed as described elsewhere (18). Briefly, genomic DNA was first amplified by PCR using two different oligonucleotide pairs. One of the PCR fragments was then digested with the restriction enzyme BstNI. Absence of the restriction site indicated a specific point mutation. The other fragment was used as a template for two allele specific PCR reactions: one with a wild-type specific primer pair and the other with a mutation specific primer pair, which detected the second point mutation studied. The final samples were separated electrophoretically on a 1.8% agarose gel and visualized by UV-irradiation using ethidium bromide staining.

The CYPIAl genotyping analysis on lymphocyte DNA was performed in principle as described by Kawajiri and co-workers (17) and given in detail elsewhere (19).

Statistical Analyses

The chi-square (contingency tables) test with Yates’ correction was used to test the associations between the different genotypes and cancer incidence, as well as other clinical parameters.

Results and Discussion

The electrophoresis results of the combined PCR-genotyping of the CYPI2D6 gene and the RFLP analysis of the CYPIAl gene are shown in Figure 1.

The distribution of different CYPI2D6 genotypes among the lung cancer patients and voluntary blood donor controls is shown in Table 1. All the lung cancer patients studied were assessed as either homozygous for two normal alleles or heterozygous for one normal and one mutant allele (i.e., they all had the EM genotype). Except for the lack of lung cancer patients with a PM genotype, the allelic frequencies in the two populations were quite similar. The percentage of observed PMs in the control population was 3.2%, which fell well within the range obtained previously in Finnish phenotypic studies (20). A PCR-based CYPI2D6 genotyping approach similar to ours was applied in a recent study by Daly and co-workers and yielded a good correlation between the debrisoquine metabolic ratio and genotype (21).

The RFLP polymorphism in the CYPIAl gene was detected by the presence or absence of the MspI site located in the 3’ end of the gene (17) resulting in C2 and C1 alleles, respectively. The distribution of the different CYPIAl genotypes in the three populations is shown in Table 1. The results indicate no difference in the genotypic frequencies between lung cancer and control populations. Furthermore, neither of the studied genotypes revealed any differences between the various histological subtypes of lung cancer (18,19).

These conclusions on the CYPIAl genotypes are in contrast to the results of Kawajiri et al. (17) who showed a statistically significant association between the rare C2 allele and certain types of lung cancer. In the Japanese study, frequencies of genotypes among lung cancer patients were significantly different from those among healthy controls. In relation to histological types of lung cancer, the squamous cell carcinoma gave the most
Table 1. Distribution of CYP2D6 and CYP1A1 genotypes.

| CYP2D6                          | EM | HEM | PM |
|---------------------------------|----|-----|----|
| Lung cancer patients (n = 77)   |    |     |    |
| Squamous cell carcinoma (n = 39) |    |     |    |
| Adenocarcinoma (n = 30)         |    |     |    |
| Small cell carcinoma (n = 5)    |    |     |    |
| Large cell carcinoma (n = 3)    |    |     |    |
| Healthy controls (n = 94)       |    |     |    |
| CYP1A1                          |    |     |    |
| Lung cancer patients (n = 74)   |    |     |    |
| Squamous cell carcinoma (n = 38) |    |     |    |
| Adenocarcinoma (n = 28)         |    |     |    |
| Small cell carcinoma (n = 5)    |    |     |    |
| Large cell carcinoma (n = 3)    |    |     |    |
| Patients with other lung diseases (n = 30) | |     |    |
| Healthy controls (n = 118)      |    |     |    |

Abbreviations: EM, extensive-metabolizer genotype; HEM, heterozygous extensive-metabolizer genotypes; PM, poor-metabolizer genotypes.

remarkable deviation from the genotype frequencies in the healthy controls; about 5-fold risk of C2C2 type was calculated for this smoking related type of lung tumor. Recently, the Japanese group also reported that the smokers with the susceptible genotype were at a remarkably high risk at a low dose level of cigarette smoking and that the difference between genotypes was reduced at high dose levels (22).

In contrast to the Japanese findings, in a similar study concerning Norwegian lung cancer patients, no statistically significant differences in allelic frequencies or distribution of the CYP1A1 genotypes were seen (23). Furthermore, the proportion of the rare genotype C2C2 was very small compared to the Japanese population.

In the present preliminary study, which included relatively small study populations, the association of CYP1A1 and CYP2D6 genes with individual susceptibility to lung cancer could neither be confirmed nor contradicted. It is

Figure 1. Examples of the electrophoresis results from the CYP2D6 and CYP1A1 genotyping analyses. (A) The six different genotypes from CYP2D6 PCR-based analysis. Genotypes are resolved by combining the information from the three parallel lanes; EM, homozygous extensive metabolizer genotype; HEM, heterozygous EM genotypes; PM, PM genotypes. (B) Autoradiogram of CYP1A1 MspI restriction fragment length polymorphism analysis showing the genotypes C2C2 (lane 2), C1C2 (lanes 5–7, 11, 12) and C1C1 (lanes 1,3,4,8–10,13,14).
evident that more studies involving larger study populations from various ethnic subgroups are needed to draw any conclusions concerning the possible association between polymorphisms in the cytochrome P450 gene superfamily and lung cancer risk.

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