

Allelic Frequency of a *p53* Polymorphism in Human Lung Cancer

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Abstract

p53 is a tumor suppressor gene that is mutated in diverse tumor types. Here we report the frequencies of common polymorphic variants at codon 72 of the *p53* gene in germline DNA of lung cancer cases and controls as determined by a polymerase chain reaction strategy. The observed allelic distribution was found to be significantly different between African-Americans and Caucasians in this U.S. population. The frequency of polymorphic variants was similar in lung cancer cases and controls after adjustment for race. However, among lung cancer patients the proline variant at codon 72 was in excess in adenocarcinoma patients by comparison with other histologies.

Introduction

Mutations in the *p53* gene have been identified in many forms of human cancer and appear to be important genetic events in cancer of the lung (1–7). Evidence is accumulating which suggests that specific mutations may be characteristic of tumor type or environmental exposure (7–10). Germline mutations in *p53* have also been reported to be associated with the Li-Fraumeni cancer family syndrome and other inherited susceptibilities to cancer (11–13).

A common polymorphism at codon 72 in the *p53* gene can be found by restriction enzyme analysis (AccII). The frequency of the major, restriction site present (G CGC) allele compared to that of the minor, restriction site absent (G CCC) allele has been reported to be approximately 0.65:0.35 in two independent studies on healthy Caucasian subjects (14, 15). The gene products of the two possible polymorphic variants differ by the presence of either arginine (major allele), a large, polar amino acid residue, or proline (minor allele), a small, nonpolar amino acid residue (16). Taken together with information that implicates *p53* mutations in human lung cancer, this suggested the possibility that the *p53* geno-

type might be influential for an individual's risk of cancer. We hypothesized therefore that the presence of the minor allele would be overrepresented in lung cancer cases compared to controls.

In order to test this hypothesis, germline DNA samples (extracted from peripheral blood lymphocytes) of lung cancer cases and controls were analyzed using PCR²/restriction analysis (AccII) methodology. With regard to the study design, since it is known that tobacco smoking is the major etiological factor in lung cancer development, that lung cancer occurs primarily in older people (age usually greater than 40 years), and that racial differences for lung cancer incidence have been noted (17), the study was carefully controlled for age, smoking, and race.

Materials and Methods

Lung Cancer Cases and Controls. Previously, we have reported the results of a lung cancer case-control study conducted on subjects accrued in two hospitals in the Baltimore-Washington metropolitan area between 1985 and 1989 (18–22). Briefly, the cases had a diagnosis of lung cancer confirmed by histological review, and these patients participated prior to radiation or chemotherapy. Two control groups were chosen; one comprised patients with chronic obstructive pulmonary disease, and the other consisted of patients with malignancies at anatomical sites other than the lung or urinary bladder. Cases and controls were closely matched with respect to age (mean for cases, 64 years; means for control groups, 62 years for individuals with chronic obstructive pulmonary disease and 61 years for individuals with cancer at anatomical sites other than the lung or bladder), race, and smoking history (average pack-years for cases, 57.4; controls, 57.1). Further details of study design, including exclusion criteria, have been described by Caporaso *et al.* (18). From the total population of 188 subjects originally studied, 150 were analyzed for the *p53* polymorphism at codon 72 based on the availability of DNA.

Laboratory Assay. A facile PCR/restriction digest-genotyping test was developed based on previous reports (14, 15). The methodology used here, however, included an important modification (23): the primer flanking codon 72 in the 5' region contained a single base pair mismatch resulting in the formation of a new AccII restriction site between exons 2 and 3 (an A→C change at position 11863 of *p53*) as an internal control for completion of digestion. High-molecular-weight DNA was isolated from WBC of subjects accrued in a case-control study of lung

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² The abbreviation used is: PCR, polymerase chain reaction.

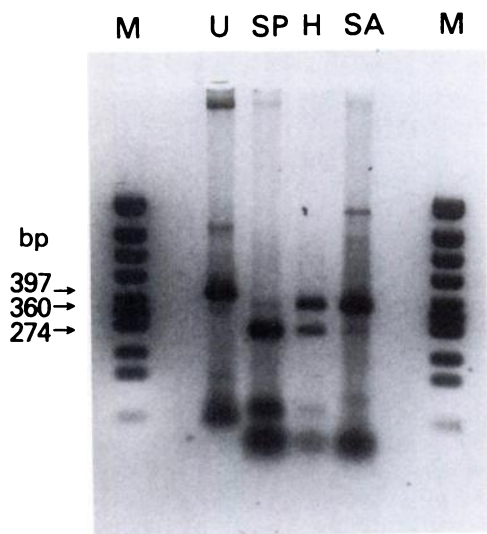


Fig. 1. PCR/restriction enzyme analysis of human genomic DNA for the *AcclI* polymorphism in exon 4 of *p53*. Lane *U*, full-length (397 base pair) undigested PCR product of amplification with primers 1 and 2 (undigested control). Lanes *SA*, *SP*, and *H*, results of *AcclI* digestion for three individuals who were determined to be homozygous *AcclI* restriction site absent (CCC or proline genotype, single 360-base pair band), homozygous *AcclI* restriction site present (CGC or arginine genotype, single 274-base pair band), or heterozygous (360 and 274-base pair bands), respectively. Note that the undigested control is 37 base pairs longer than all of the digested materials because of the presence of the *AcclI* restriction site introduced by primer 1 and that the shortest *AcclI* digestion products have migrated out of the gel. Lanes *M*, are λ phage DNA cleaved with *HaeIII*.

cancer. DNA samples were amplified for 30 cycles using a standard protocol (8) with either primer 1 and 2 (below) or by a heminested strategy using primers 1 and 2 followed by a second round of amplification with primers 1 and 3 (primer 1 = 5'-CCCCAACCCAGCCCCCT-AGCAGAGACCTGTGGGACGCG-3' [mismatch position underlined]; primer 2 = 5'-TGTCATCTTCTGTCCCTTCCCAGA-3'; primer 3 = 5'-ACACCGGCGGCCCC-TGCACCA-3'). The resulting PCR products were digested to completion with *AcclI* and analyzed by agarose gel electrophoresis (2% in Tris/borate buffer, pH 8.3).

Results

The results of PCR amplification of genomic DNA from lung cancer case-control study subjects using primers specific for exon 4 of *p53* and subsequent restriction enzyme (*AcclI*) digestion are shown in Fig. 1. In this figure, Lane *U* contains PCR-amplified but undigested control material, Lane *SP* contains a sample derived from an *AcclI* restriction site present homozygote (arg/arg), Lane *H* contains a sample derived from a heterozygote (pro/arg), and Lane *SA* contains a sample derived from an *AcclI* restriction site absent homozygote (pro/pro). The results of dideoxy chain termination DNA sequencing of the PCR products from a small subset of samples to confirm their authenticity were consistent with the restriction analysis (data not shown).

The PCR analysis with restriction enzyme digestion was performed on 150 study subjects (78 cases and 72 controls), and the results are given in Table 1 and Fig. 2. There was no association of the *p53* genotype with a

Table 1 Distribution of *p53* alleles by race

Study group	pro/pro ^a	arg/pro	arg/arg
Blacks	26 (37) ^b	34 (49)	10 (14)
Whites	9 (11)	36 (45)	35 (44)
Lung cancer	16 (21)	38 (49)	24 (31)
COPD ^c	8 (17)	24 (51)	15 (32)
Other cancer	11 (44)	8 (32)	6 (24)
Pooled controls ^d	19 (26)	32 (44)	21 (29)
Total	35 (23)	70 (47)	45 (30)

^a pro/pro, proline homozygote; arg/pro, heterozygote; arg/arg, arginine homozygote, where the arginine allele is the *AcclI* restriction site present variant and the proline allele is restriction site absent.

^b Numbers in parenthesis, percentages.

^c COPD, chronic obstructive pulmonary disease.

^d Pooled controls are COPD patients combined with patients having malignancies at anatomical sites other than the lung or urinary bladder.

diagnosis of lung cancer ($\chi^2 = 0.73$; $P = 0.69$). However, the observed allelic distribution was found to be significantly different between African-Americans and Caucasians ($\chi^2 = 21.6$; $P < 0.0001$). Thus, the most common allele in African-Americans is the restriction site absent allele (0.39:0.61), whereas the analysis of samples obtained from Caucasians was similar to the frequencies previously reported (0.65:0.35) (14, 15).

The data were further analyzed for differences among histological types of lung cancer and for the different control groups (Fig. 2). A tendency toward increasing frequencies of the proline variant (*AcclI* restriction site absent allele) was noted in lung cancer patients with adenocarcinoma compared to other histologies (squamous cell, small cell, and large cell carcinomas). This tendency was statistically significant in Caucasians ($\chi^2 = 10.40$; $df = 2$; $P = 0.006$; $n = 42$) but not African-Americans ($\chi^2 = 1.44$; $df = 2$; $P = 0.49$; $n = 36$). When the frequency of the proline variant in adenocarcinoma was compared to control subjects for Caucasians only a nonsignificant association was seen ($\chi^2 = 4.15$; $df = 2$; $P = 0.11$). The Mantel-Hanzel summary statistic also indicated a nonsignificant association ($\chi^2 = 1.52$; $df = 2$; $P = 0.22$). There were no associations between the *p53* genotype and lung cancer risk factors (age, family history, gender, smoking, asbestos exposure, or debrisoquine metabolic phenotype) other than race. Thus, multivariate control for these factors did not result in any evidence of risk associated with the *p53* polymorphism. Similarly, controlling for these variables did not alter the strongly significant ($P = 0.0001$) association between the *p53* polymorphism and race.

Discussion

No association was found between the allelic frequencies of *p53* and lung cancer, in either Caucasian or African-American study subjects. However, a tendency was found for the proline allele to be overrepresented in lung adenocarcinoma patients compared to other histological types of lung cancer, irrespective of race, although the association reached statistical significance only in Caucasians. These data need to be interpreted cautiously since this association was not significant when adenocarcinoma cases were compared with control subjects ($\chi^2 = 4.15$; $df = 2$; $P = 0.11$). Therefore, independent testing in a separate sample set is required.

Data did show that the proline variant of the *AcclI* polymorphism in the coding region of *p53* is more fre-

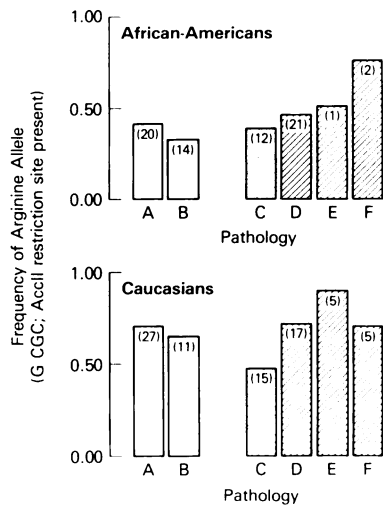


Fig. 2. Frequency of polymorphic variants of exon 4 (codon 72) of the *p53* gene locus in lung cancer cases and controls. A, chronic obstructive pulmonary disease; B, nonpulmonary cancer; C, adenocarcinoma; D, squamous cell carcinoma; E, large cell carcinoma; F, small cell carcinoma. Numbers in parentheses, actual numbers of study subjects. □, adenocarcinoma; ▨, nonadenocarcinoma; □, controls. Data are expressed as the frequency of the arginine variant as opposed to the proline variant where the sum of the frequencies for each allele is 1.00.

quent in African-Americans than in Caucasians. This racial difference was highly significant and adds to the evidence of substantial genetic differences by race with respect to genes thought to be responsible for some aspects of carcinogenesis (19–21, 24). Tobacco smoke is the major etiological agent in lung cancer; however, not all individuals that smoke cigarettes develop lung cancer, while some individuals with little or no exposure do develop lung cancer. These observations suggest that other factors, both etiological (e.g., radon) and genetic, may be important in the determination of lung cancer risk. The racial variations in allelic distribution that have been observed for polymorphisms in protooncogene (*L-myc* and *HRAS-1*) and tumor suppressor gene (*p53*) loci indicate that controlling for racial variation as a confounding factor in epidemiological investigations of these factors is an important facet of study design.

A third polymorphic variant in codon 72 was originally described by Matlashewski *et al.* (16) to be cysteine (TGC). By using a mismatch primer (C→A change introduced in codon 71) it should be possible to create a *Bsp*MI restriction site (ACCTGC) in the PCR product. With this approach, however, no cysteine variants were detected in this study. The most likely explanation for these results is the probability that the cysteine variant arose as an artifact of the original cloning procedure (16).

The current data are interesting because of the ethnic variation and possible association with adenocarcinoma. Therefore, this polymorphism is worthy of further study, particularly in view of the significant difference between various ethnic groups, especially since the medical and scientific data base on minorities is currently lacking.

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