Short Communication

Alpha₁-Antitrypsin Deficiency Allele Carriers among Lung Cancer Patients

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Abstract

Lung cancer (LC) and chronic obstructive pulmonary lung diseases (COPDs; including emphysema and chronic bronchitis) share a common etiology. Despite the known associations of alpha₁-antitrypsin deficiency (α₁AD) with COPD and COPD with LC, few studies examined the association of α₁AD alleles and LC. We hypothesize that heterozygous individuals who carry a deficient allele of the α₁AD gene Pi (protease inhibitor locus) are at an increased risk of developing LC. The Pi locus is highly polymorphic with >70 variants reported. There are at least 10 alleles associated with deficiency in alpha₁-antitrypsin. Using an exact binomial test, we compared the α₁AD carrier rate in 260 newly diagnosed Mayo Clinic LC patients to the reported carrier rate in the general population. The α₁AD carrier status, determined by isoelectric focusing assay, was examined with respect to the history of cigarette smoking, COPD, and histological types. Thirty-two of the 260 patients (12.3%; 95% confidence interval, 8.6 –16.9%) had allele S, 6 had allele Z, and 2 had allele L. Patients who never smoked cigarettes were three times more likely to carry a deficient allele (20.6%; P = 0.008), although smokers had a higher carrier rate (11.1%; P = 0.025) when compared with the 7% rate. Patients with squamous cell or bronchoalveolar carcinoma had a significantly higher carrier rate than expected (15.9% and 23.8%, P ≤ 0.01, respectively). Our preliminary findings suggest that individuals who carry an α₁AD allele may have an increased risk for developing LC, specifically squamous cell or bronchoalveolar carcinoma.

Introduction

An unresolved paradox of LC is that, although a majority of the patients are tobacco users, only a minority of long-term smokers develop LC. In addition to cigarette smoking, another well-known risk factor for LC is COPD, which includes emphysema and chronic bronchitis (1). COPD not only increases LC risk in both smokers and nonsmokers, but also shares common risk factors with LC (1–4). Both diseases are strongly associated with tobacco use (1, 5), and both aggregate in families (4–6). Individuals who are homozygous for the α₁AD gene (7) or who are heterozygous for this gene (α₁AD carriers) are predisposed to the development of COPD (8–10). However, it is not known whether α₁AD individuals and carriers are at increased risk of LC.

α₁AT, a secretory glycoprotein produced in the liver, is a protease inhibitor and neutralizes the effects of proteases in several organ systems, mainly the lung. It is believed that COPD develops in α₁AD individuals as a result of an imbalance between neutrophil elastase (a protease) and α₁AT in lung tissue (8, 11, 12). This imbalance could be due to an excess of elastase and/or a lack of functional α₁AT (8, 13). Normal plasma α₁AT concentration or level is 110–200 mg/dl, and α₁AD individuals have α₁AT levels ranging from 0–60 mg/dl (7, 14). The α₁AT level is marginally normal in those who are heterozygous for the deficient alleles (70–110 mg/dl; Refs. 14 and 15). Tobacco smoke disturbs the balance between protease and protease inhibitor activity in lung tissue by stimulating neutrophils to secrete more elastase (16) and inactivating α₁AT (17, 18), thereby leading to elastolytic destruction of lung tissue (19). Because of debilitating consequences (7, 14, 19), individuals with known α₁AD usually have minimal or no tobacco smoke exposure (20). α₁AD carriers do not normally suffer from severe α₁AD-related diseases; however, they may be especially vulnerable to tobacco smoke-related diseases. Whether an individual with elastolytically destroyed lung tissue is more susceptible to respiratory carcinogens has not been properly evaluated. As an initial step to test this hypothesis, we attempt to answer the following question: Are LC patients more likely to carry α₁AD alleles than the general population?

Materials and Methods

Study Subjects. Patients were selected from an ongoing comprehensive LC study at the Mayo Clinic in Rochester, Minnesota. The goals of this study are to examine the roles of genes and environmental exposures in LC etiology, to search for susceptibility and risk-modifying genes, and to identify at-risk individuals. Since March 1997, all patients who were newly diagnosed with primary LC have been identified and evaluated

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for study eligibility. Our eligibility criteria were developed to efficiently achieve our goal of a balanced case group by oversampling cases with characteristics indicative of other risk factors besides smoking: (a) all cases with a positive FH of LC in at least one first-degree relative, and all cases with a positive FH of other cancers in at least two first-degree relatives; (b) all cases who are diagnosed at age 50 or younger; (c) all cases who are lifetime nonsmokers (smoked <100 cigarettes during lifetime); (d) all cases who have uncommon tumor types; and (e) a 20% sample of those LC patients who do not meet criteria 1–4 (i.e., the sporadic group). Group 5 can serve as an internal comparison for groups 1–4 and can be used to derive a complete patient ascertainment. The sporadic cases were selected in such a way that every fifth sporadic case identified was enrolled to achieve a 20% sample of the patients in this category. The rationale for our sampling strategy is as follows: although a majority of all cases are cigarette smokers, LC presents as a very heterogeneous disease with regard to clinical presentation, histopathological features, age at diagnosis, and FH of cancer. On average, one-half of the patients are so-called “sporadic” cases who seem to be typically smoking-related, develop a common type of LC after 50 years of age, and have no significant FH of lung or other cancer. Common types of LC include adenocarcinoma, squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and unspecified non-small cell LC.

New patients were ascertained daily from our computerized pathology reporting system, which identifies approximately 95% of all LC cases seen at our institution. The remaining 5% of the patients were identified directly from the patient-care physicians. Medical records for each patient were reviewed to determine study eligibility, except for a small number of patients who denied research authorization for review of their medical records (<1%). After informed consent, each patient was interviewed by a certified genetic counselor (K. A. W.) for complete FH (a five-generation pedigree). Patients who were classified as nonsmokers were confirmed at the interview, and a detailed passive smoking history was obtained. From each consenting patient, a 28-ml blood sample and environmental exposure information (tobacco consumption, dietary pattern, and occupational exposures) were requested for study.

Due to the dynamic nature of an ongoing study, this report presents preliminary results based on cases identified during the first 9 months of our study. Between March and November 1997, 597 eligible patients were identified. Among these, 400 (67.3%) were interviewed and 68 (11.4%) declined. Three hundred eighty-eight of the interviewed patients (97%) agreed to donate blood. 302 blood samples have been received, and α₅AT allele types were tested on 260 patients in the study period defined for this early report. Because the α₅AD allele frequency has been well documented worldwide and varies greatly from Caucasians to other populations (7, 21–23), only patients of European origin were included in the current report. Among the 42 patients (i.e., the difference between the 302 and 260), for whom we have collected blood samples but were not included in this early report, 3 were non-Caucasian of European origin and 39 did not have a pathological report from our institution.

Data Collection. Information abstracted from the patients’ medical records includes pathology, clinical staging, treatment, history of previous diseases, lifestyle (tobacco, alcohol, and coffee use), education, occupation, a brief FH, demographics, and follow-up data. History of COPD was based on explicit diagnosis (24) or abnormal pulmonary function tests (25) that were recorded in the patients’ medical histories. During the patient interview, a five-generation pedigree was constructed for each patient. Data collected for each relative in the pedigree included: vital status and health history concerning malignant and nonmalignant diseases (age at diagnosis and/or cause of death). Information on tobacco use and major occupation was collected for all first-degree relatives. α₅AT Allele Determination and Plasma Concentration Measurement. α₅AD is a common autosomal recessive disorder caused by mutations of the protease inhibitor locus Pi, located on chromosome 14q32.1 (7, 21). Pi is highly polymorphic with >70 variants reported (7, 26, 27). Each variant is designated by a letter corresponding to the migration of the α₅AT protein in isoelectric focusing assay, the standard clinical diagnostic test for more than 20 years (28–33). M (including subtypes M₁, M₂, and M₃) is the most common, with most normal individuals being homozygous for this allele (designated as MM or PiMM with the subtypes, e.g., M₁M₁). Of the variants that lead to a deficiency of α₅AT, only Z and S alleles are common. Uncommon deficient alleles include I, MₛAlternate, MᵢAlternate, null, and other rare alleles (7). Isoelectric focusing assay, performed in the Mayo Clinic Protein and Immunopathology Laboratory (33, 34), was used to type α₅AT alleles, and the concentration of plasma α₅AT was determined by nephelometry using standard protocol by Beckman Instruments, Inc. (35).

Data Analysis. An exact binomial test (36) was used to compare the α₅AD carrier rate among LC patients with the expected frequency of 7%. An observed to expected ratio was calculated. Although Pi allele frequencies vary substantially across geographic regions and ethnic groups in Europe, they are fairly homogeneous in the United States white population (22, 23, 37, 38). The Z allele is found in 1–2%, and the S allele in 2–4% of all Caucasians of European descent, but <1% for both alleles combined in Asians and Africans (7, 8, 23, 39). Previous studies in Minnesota populations reported a prevalence of 1.4% for the Z allele and 2.3% for the S allele (37–39). Assuming Hardy-Weinberg equilibrium (40), the proportion of heterozygous individuals is estimated at 7%. The J allele, which causes moderate α₅AD (60–70% of normal level), is very rare with a frequency of <0.003 in United States whites (38, 41). Results were also stratified by history of COPD and tobacco smoking (coded as binary variables). Smoking status was divided into never- and ever-smoked in this analysis. χ² statistics were used in comparing carriers to noncarriers for smoking and COPD history, and the Wilcoxon rank sum test was used to compare ages and α₅AT levels of the carriers and noncarriers (42). The 95% CIs are exact intervals based on Feller (43).

Results

Our study group, as shown in Table 1, included 164 men and 96 women with a gender ratio of 1.7 males to 1 female and a mean age at diagnosis of 63.27 (± 12.67) years. Eligible patients not included in the current analysis did not differ significantly with respect to gender, mean age at diagnosis, study eligibility criteria, or histological type of tumor. Thirty-two (12.3%) of the 260 patients studied were found to be α₅AD carriers (19 males, 13 females). When compared with the 7% carrier rate expected for the United States white population, the overall 12.3% carrier rate in our sample was significantly higher (P = 0.002). The carrier rate among female patients (13.5%) was not significantly different from that among male patients (11.6%), each higher than expected (P < 0.05). Patients who never smoked cigarettes were three times more likely to carry a deficient allele (20.6%; P = 0.008), although smokers had a higher carrier rate (11.1%; P = 0.025) when compared with the 7% rate. FH of LC or other cancers did not differ in the α₅AD carrier rate from the
sporadic group in our study sample (12.8%, 13.3%, and 10.9%, respectively).

Twenty-four (75.0%) of the 32 α1AD carriers had an S allele, 6 (18.8%) had a Z allele, and 2 (6.3%) had an I allele. Among the 228 noncarriers, 125 (54.8%) were homozygous M1M1, 81 (35.5%) were heterozygous of M1 and M2 or M3, and 21 (9.2%) were homozygous or double heterozygous of M2 or M3. We have also observed a GM1 (0.4%) allele type that is one of the normal, but rare, variants of the Pi locus (44). Mean levels of serum α1AT were 198.1 mg/dl for carriers and 265.6 mg/dl for noncarriers. This difference is highly significant (P < 0.0001), albeit both are considered normal.

Carrier rates were compared with respect to age at diagnosis, history of COPD, and cigarette smoking (Table 2). In the older age group, a significantly higher carrier rate was seen in smokers (12.0%) and marginally significant in nonsmokers (20%), compared with the expected rate. In contrast, a higher carrier rate was not observed in the younger age group. Prior history of COPD (16 of 32 or 50.0% versus 91 of 225 or 40.4%; P = 0.012) and cigarette smoking (25 of 32 or 78.1% versus 201 of 428 or 47.5%, P = 0.015) were not significantly different between the carriers and noncarriers, respectively. Also shown in Table 2 are the significantly higher carrier rate in patients who had a COPD history (15.0%; P = 0.003). Among patients without a COPD history, only nonsmokers had a significantly higher carrier rate (18.8%; P = 0.022).

We further examined the α1AD carrier rate by histological type of the tumor (Table 3). There were 126 (48.5%) patients with adenocarcinoma, 63 (24.2%) with squamous cell carcinoma, 7 (2.7%) with small cell and 6 (2.3%) with large cell carcinoma, 16 (6.2%) with carcinoid, 11 (4.2%) with undifferentiated non-small cell carcinoma, and 6 (2.3%) with other carcinomas. Fourteen of the 126 cases with adenocarcinoma were α1AD carriers (11.1%; P = 0.113) and 10 of the 63 patients with squamous cell carcinoma were carriers (15.9%; P = 0.012). Although the carrier rate in adenocarcinoma was not significantly higher than the expected 7% rate, a much higher carrier rate, 5 of 21 (23.8%, P = 0.013), was found in BAC, a subgroup of adenocarcinoma. The carrier rate ranged from 0–16.7% for the remaining cell types, but none was found statistically significant. This could be due to a limited number of patients in each group.

After stratifying patients by cigarette smoking (also in Table 3), we found the higher carrier rate was especially evident (row 6) among the 8 patients with BAC who were nonsmokers. A subgroup of adenocarcinoma (second row from bottom) who were smokers (16.1%; P = 0.011). In contrast, neither smokers nor nonsmokers of the remaining adenocarcinoma group (non-BAC) were more likely to carry the α1AD allele than expected.

### Discussion

Our preliminary results demonstrate that LC patients, both smokers and nonsmokers, were more likely to carry an α1AD allele than the general United States white population. Specifically, patients with squamous cell or BAC were much more likely to be carriers than expected. An association between LC and α1AD carrier state has not been reported previously.

Our results were based on an expected carrier rate of 7%, which was the most commonly quoted Pi allele frequency for the United States white population (7, 21). These allele fre-
Second, specific alterations in the Pi gene might directly predispose an individual to higher LC risk. The Pi gene is highly polymorphic and its functional domains are now characterized (27, 47). The alterations causing Z and S alleles are in exons 5 and 3, respectively. Interestingly, in exon 1b of the Pi gene, there are two binding sites capable of interacting with the c-jun (AP-1) proto-oncogene products (14, 48, 49). The role of the combinations of Pi gene mutations and/or c-jun (AP-1) activities should be studied.

We would expect to observe a stronger association between smokers and α1AD carriers than that in nonsmokers based on the first of our postulated mechanisms (the presence of lung tissue damage and carcinogen exposure). For nonsmokers, when sample size increases substantially, we could further test whether passive smoking and undiagnosed mild lung dysfunctions (as shown in pulmonary function tests) are significant risk factors. However, it was not surprising to observe a stronger association between nonsmokers and α1AD carriers than that in smokers based on our alternative hypothesis that there may be an interaction between the Pi locus and a proto-oncogene, c-jun. It is also possible that the role of the deficient Pi allele differs in smokers from that in nonsmokers in LC development.

Although within the normal range, the mean serum α1AT level was significantly lower in carriers than in noncarriers. It is known that α1AT increases in several conditions, including malignancies, and this elevation in α1AT concentration has been considered a physiological reaction. Our findings were consistent with the reported literature and suggested a compromised reactive α1AT level among α1AD carriers compared with the noncarriers. It is not clear whether a proper reactive response might be part of the protective mechanism in certain adverse situations such as malignancies.

We did not observe a single patient who was homozygous for α1AD alleles. The relative rarity of homozygous individuals in United States whites (1/2500; Refs. 7 and 21), coupled with their associated higher mortality (50), reduced exposure to tobacco smoke (20), and the generally late onset of LC, are explanations.

In conclusion, markers for genes coding organ- or tissue-specific functional products (for example, protease and protease inhibitors) and their interaction with environmental exposures have not been adequately studied in LC etiology. Our findings suggest that α1AD carriers may have an increased risk for developing squamous cell or BAC of the lung. We hope that our findings will stimulate further investigations in searching for genetic markers and their roles in LC development.

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