Short Communication

Alpha$_1$-Antitrypsin Deficiency Allele Carriers among Lung Cancer Patients[1]

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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 alpha$_1$-antitrypsin deficiency (α_1AD) with COPD and COPD with LC, few studies examined the association of α_1AD alleles and LC. We hypothesize that heterozygous individuals who carry a deficient allele of the α_1AD 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 alpha$_1$-antitrypsin. Using an exact binomial test, we compared the α_1AD carrier rate in 260 newly diagnosed Mayo Clinic LC patients to the reported carrier rate in Caucasians in the United States (7%). α_1AD 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%) carried an α_1AD allele, which was significantly higher than expected ($P = 0.002$). Twenty-four of the 32 carriers had allele *S*, 6 had allele *Z*, and 2 had allele *I*. 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%, $P \leq 0.01$, respectively). Our preliminary findings suggest that individuals who carry an α_1AD allele may have an increased risk for developing LC, specifically squamous cell or bronchoalveolar carcinoma.

Introduction

An unresolved paradox of LC[3] 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 *α_1AD* gene (7) or who are heterozygous for this gene (α_1AD carriers) are predisposed to the development of COPD (8–10). However, it is not known whether α_1AD individuals and carriers are at increased risk of LC.

α_1AT, 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 α_1AD individuals as a result of an imbalance between neutrophil elastase (a protease) and α_1AT in lung tissue (8, 11, 12). This imbalance could be due to an excess of elastase and/or a lack of functional α_1AT (8, 13). Normal plasma α_1AT concentration or level is 110–200 mg/dl, and α_1AD individuals have α_1AT levels ranging from 0–60 mg/dl (7, 14). The α_1AT 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 α_1AT (17, 18), thereby leading to elastolytic destruction of lung tissue (19). Because of debilitating consequences (7, 14, 19), individuals with known α_1AD usually have minimal or no tobacco smoke exposure (20). α_1AD carriers do not normally suffer from severe α_1AD-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 *α_1AD* 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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[3] The abbreviations used are: LC, lung cancer; COPD, chronic obstructive pulmonary diseases; α_1AT, alpha$_1$-antitrypsin; α_1AD, alpha$_1$-antitrypsin deficiency; BAC, bronchoalveolar carcinoma; CI, confidence interval; FH, family history.

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 α_1*AT* allele types were tested on 260 patients in the study period defined for this early report. Because the α_1*AD* 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.

α_1-*AT* Allele Determination and Plasma Concentration Measurement. α_1AD 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 α_1AT protein in isoelectric focusing assay, the standard clinical diagnostic test for more than 20 years (28–33). M (including subtypes M_1, M_2, and M_3) is the most common, with most normal individuals being homozygous for this allele (designated as MM or *Pi*MM with the subtypes, *e.g.*, M_1M_1). Of the variants that lead to a deficiency of α_1AT, only *Z* and *S* alleles are common. Uncommon deficient alleles include *I*, M_{Malton}, $M_{Pittsburgh}$, null, and other rare alleles (7). Isoelectric focusing assay, performed in the Mayo Clinic Protein and Immunopathology Laboratory (33, 34), was used to type α_1*AT* alleles, and the concentration of plasma α_1AT was determined by nephelometry using standard protocol by Beckman Instruments, Inc. (35).

Data Analysis. An exact binomial test (36) was used to compare the α_1AD 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-Weinburg equilibrium (40), the proportion of heterozygous individuals is estimated at 7%. The *I* allele, which causes moderate α_1AD (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. χ^2 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 α_1AT 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 α_1AD 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 α_1AD carrier rate from the

Table 1 Basic characteristics of patients in Mayo Comprehensive Lung Cancer Study, March–November 1997

	Cases included in current report		All eligible cases identified	
	n	%	*n*	%
Gender				
Male	164	63.1	340	57.0
Female	96	36.9	257	43.0
Study eligibility criteria[a]				
FH of LC	78	30.0	155	26.0
FH of other cancer	75	28.8	165	27.6
Age ≤50 years at diagnosis	51	19.6	90	15.1
Lifetime nonsmoker	34	13.1	88	14.7
Uncommon tumor type	54	20.8	166	27.8
Sporadic	46	17.7	70	11.7
Place of residence				
Minnesota	94	36.1	199	33.3
Other midwestern states[b]	129	49.6	319	53.4
Other states or Canada	37	14.2	79	13.2
Total	260		597	

[a] Patients meeting multiple criteria were counted in each category.
[b] Includes Wisconsin, Michigan, Iowa, Illinois, Indiana, Ohio, North Dakota, South Dakota, Nebraska, Kansas, and Missouri.

Table 2 Age at diagnosis, history of COPD, and smoking status among 260 primary lung cancer cases

Age at diagnosis and history of COPD[a]	Number of cases (% of group total)	α_1AD carriers		
		n	%	95% CI
All LC cases	260 (100)	32	12.3	8.6–16.9
Smokers	226 (86.9)	25	11.1	7.3–15.9
Nonsmokers	34 (13.1)	7	20.6	8.7–37.9
Diagnosed >50 years	209 (100)	27	12.9	8.7–18.2
Smokers	184 (88.0)	22	12.0	7.7–17.5
Nonsmokers	25 (12.0)	5	20.0	6.8–40.7
Diagnosed ≤50 years	51 (100)	5	9.8	3.3–21.4
Smokers	42 (82.7)	3	7.1	1.5–19.5
Nonsmokers	9 (17.3)	2	22.2	2.8–60.1
With COPD history	107 (100)	16	15.0	8.8–23.1
Smokers	105 (98.1)	15	14.3	8.2–22.5
Nonsmokers	2 (1.9)	1	50.0	1.3–98.7
No COPD history	150 (100)	16	10.7	6.2–16.7
Smokers	118 (78.7)	10	8.5	4.1–15.0
Nonsmokers	32 (21.3)	6	18.8	7.2–36.4

[a] Of the 260 patients, missing COPD data on 3 patients.

sporadic group in our study sample (12.8%, 13.3%, and 10.9%, respectively).

Twenty-four (75.0%) of the 32 α_1AD 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 M_1M_1, 81 (35.5%) were heterozygous of M_1 and M_2 or M_3, and 21 (9.2%) were homozygous or double heterozygous of M_2 or M_3. We have also observed a GM_1 (0.4%) allele type that is one of the normal, but rare, variants of the *Pi* locus (44). Mean levels of serum α_1AT 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%; $P = .305$) and cigarette smoking (25 of 32 or 78.1% *versus* 201 of 228 or 88.2%, $P = 0.115$) were not significantly different between the carriers and noncarriers, respectively. Also shown in Table 2 is 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%; $P = 0.022$).

Table 3 α_1AD carrier rate by histologic type and by cigarette smoking history

Histologic type and smoking	Number of cases	α_1 AD carriers		
		n	%	95% CI
Adenocarcinoma	126	14	11.1	6.2–18.0
Smokers	106	10	9.4	4.6–16.7
Nonsmokers	20	4	20.0	5.7–43.7
BAC	21	5	23.8	8.2–47.2
Smokers	13	2	15.4	1.9–45.5
Nonsmokers	8	3	37.5	8.5–75.5
Non-BAC-adenocarcinoma	105	9	8.5	4.0–15.6
Smokers	93	8	8.6	3.8–16.3
Nonsmokers	12	1	8.3	0.2–38.5
Squamous cell	63	10	15.9	7.9–27.3
Smokers	62	10	16.1	8.0–27.7
Nonsmokers	1	0	0	

We further examined the α_1AD 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 α_1AD 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 (37.5%, $P = 0.015$) and in the 62 patients with squamous cell carcinoma (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 $\alpha_1 AD$ allele than expected.

Discussion

Our preliminary results demonstrate that LC patients, both smokers and nonsmokers, were more likely to carry an α_1AD 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 α_1AD 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-

Table 4 Reported *S* and *Z* allele frequencies in selected populations

Population	Sample size	% *S* allele	% *Z* allele	% either
United States white populations[a]	1928	3.45	1.09	4.54
Minnesota (23, 37, 38)	1032	2.45	1.42	3.87
Pennsylvania (37)	564	4.36	0.70	5.06
Utah (37)	92	7.61	0	7.61
United States whites (23)	240	4.00	1.00	5.00
Denmark (7)	900	2.20	2.30	4.50
Germany[a] (23)	1715	2.00	1.00	3.00
Great Britain (23)	926	5.00	2.00	7.00
Asia (23)	4907	0	0	0
Africa (37, 39)	843	0.60	0.40	1.00

[a] Estimated from multiple samples with allele frequency proportional to sample size of each population.

Table 5 Self-reported country of origin of grandfathers of Mayo Clinic LC patients (by %), March–November 1997

	Paternal grandfather		Maternal grandfather	
	All[a] patients	Patients in current report	All[a] patients	Patients in current report
German	37.8	39.8	31.2	32.6
English	13.0	14.1	12.7	11.6
Norwegian	12.6	12.0	10.4	12.7
Irish	7.0	7.3	9.0	10.5
Other[b]	29.6	26.7	36.7	32.6

[a] All interviewed patients.
[b] Includes more than 15 distinct ethnic groups, each less than 3% of the total.

quencies were derived from the results of multiple studies, one of which included 904 healthy blood donors in the state of Minnesota (38). As shown in Table 4, *Pi* allele frequencies vary substantially across geographic regions and ethnic groups in Europe (22, 23), but are fairly homogeneous in the United States white population (23, 37). Nonetheless, further investigation with age-, gender-, and race-matched control groups is needed.

It would be interesting to further investigate whether the α_1AD carrier rate differs within United States Caucasians. Due to the highly diverse ethnic background of United States Caucasians, it is a challenge to meaningfully group four lines of country-of-origin (ancestral background) from both sets of grandparents. Added to the complexity is the fact that 25 distinct ethnic groups were reported in our case series (which makes 25^4 combinations to consider for four grandparents). Table 5 shows the four most commonly self-reported ancestries of patients' grandfathers in our study subjects. Equally diverse information was found in patients' grandmothers. Thus, a much larger sample size is needed to detect any significant difference in α_1AD carrier rate among patients with different countries-of-origin.

Our findings differ from an earlier study by Harris *et al.* (45), which did not report any difference in *α_1AT* allele distribution between LC patients *versus* a control group in which the diagnosis of LC was excluded by sputum cytology only. The α_1AD carrier rate was 12.5% in the LC group and 15.3% in the control group. Among their 196 controls, only 53 (27.0%) had normal cytology, whereas the remainder had squamous metaplasia with or without atypia. The α_1AD carrier rate ranged from 13.2% for those with normal cytology to 27.8% for those with marked atypical squamous metaplasia (45); all were greater than the expected carrier rate in the general population. In addition, sputum cytology is a very insensitive diagnostic test for LC. Many of these "controls" may not have been free of LC. Therefore, the use of a control group with an artificially elevated carrier rate may disguise the higher carrier rate in the LC patients. By using an appropriate control group, investigators will be able to estimate the effects of known risk factors (*e.g.,* COPD and cigarette smoking) on the association between α_1AD and LC.

On the basis of our results, it is plausible to hypothesize that the damage in lung tissue resulting from an imbalance between neutrophil elastase and α_1AT is a predisposing condition for LC development. Two alternative mechanisms are postulated. First, carrying an α_1AD allele may be an indirect cause or a "paraneoplastic" marker (46) for LC. Individuals with varying degrees of lung tissue damage may have longer exposures due to air trapping, thereby increasing absorption and enhancing the action of carcinogens present in tobacco smoke. 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 Ib 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 α_1AD 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 α_1AD 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 α_1AT level was significantly lower in carriers than in noncarriers. It is known that α_1AT increases in several conditions, including malignancies, and this elevation in α_1AT concentration has been considered a physiological reaction. Our findings were consistent with the reported literature and suggested a compromised reactive α_1AT level among α_1AD 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 *α_1AD* 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 α_1AD 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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