Biochemical and Biophysical Research Communications **289,** 116–119 (2001)
doi:10.1006/bbrc.2001.5936, available online at http://www.idealibrary.com on IDEAL®

Genetic Polymorphism in Matrix Metalloproteinase-9 and Pulmonary Emphysema

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Protease-antiprotease imbalance due to genetic variation may be responsible for the development of pulmonary emphysema induced by smoking. Since matrix metalloproteinases (MMPs) have recently been suggested to play important roles in the pathogenesis of pulmonary emphysema, the association between the functional polymorphism of MMP-9 (−1562C/T) and the development of pulmonary emphysema was examined in 110 smokers and 94 nonsmokers in Japan. The T allele frequency was higher in subjects with distinct emphysema on chest CT-scans ($n = 45$) than in those without it ($n = 65$) (0.244 vs 0.123, $P = 0.02$). Logistic regression analysis demonstrated that the T allele is a risk factor for smoking-induced emphysema (odds ratio = 2.69, $P = 0.02$). DL_{CO}/VA was lower ($P = 0.02$) and emphysematous changes were more conspicuous ($P = 0.03$) in subjects with C/T or T/T ($n = 35$) than in those with C/C ($n = 75$). These results suggest that the polymorphism of MMP-9 acts as a genetic factor for the development of smoking-induced pulmonary emphysema.

Key Words: **matrix metalloproteinase-9; pulmonary emphysema; smoking; genetic polymorphism.**

Chronic obstructive pulmonary disease (COPD) is a major public health problem which is mainly caused by cigarette smoking. However, only 10–20% of smokers manifest respiratory symptoms due to impaired lung function (1). Since no effective treatment to inhibit the progression of COPD is currently available, it is of great importance to identify those subjects sensitive to smoking in the early stages of COPD, in order to decrease the worldwide burden of this disease. The introduction of α_1-antitrypsin deficiency as a risk factor for severe pulmonary emphysema has suggested that protease-antiprotease imbalance due to genetic variation may also be responsible for the development of pulmonary emphysema induced by other causes including smoking. Although various proteases and antiproteases have been identified, sensitivity to smoking has not yet been accounted for by genetic polymorphisms of these factors. Matrix metalloproteinases (MMPs) have been suggested to play roles in the pathogenesis of pulmonary emphysema (2, 3). MMP-9 and MMP-12 account for most of the macrophage-derived elastase activity in smokers (4), and the production of MMP-9 is enhanced by inflammatory mediators including cytokines (5). In addition, alveolar macrophages obtained from smokers have recently been reported to release more MMP-9 than those from nonsmokers (6), suggesting that MMP-9 is one of the candidate genes responsible for smoking-induced pulmonary emphysema. MMP-9 is also known to contribute to tissue remodeling processes in atherosclerotic plaque, aneurysmal formation, and cancer invasion as well as pulmonary emphysema (7–9). A recent study demonstrated that a polymorphism in the promoter region of MMP-9 (−1562C/T) was significantly associated with its promoter activity and the severity of atherosclerosis in patients with coronary artery disease (10). We examined the association between the genetic polymorphism of MMP-9 and the development of pulmonary emphysema in Japanese habitual smokers in the present study.

MATERIALS AND METHODS

Study population and genotyping. Applying the restriction fragment length polymorphism (RFLP) method as previously described (10), the genotypes related to −1562C/T of MMP-9 were examined in 110 Japanese smokers [104 male and 6 female, age ≥ 50 years old, lifelong cigarette consumption (CC) ≥ 10 pack-years] and 94 nonsmokers (59 male and 35 female, age < 50 years old, CC < 0.5 pack-years). Primer sequences for the PCR-RFLP were 5′-GCCTGGCACATAGTAGGCCC-3′ and 5′-CTTCCTAGCCAGCCGGCATC-3′, and the 435-bp PCR product was digested with *Bbu*I (10).

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0006-291X/01 $35.00

TABLE 1

−1562T Allele Frequency in Emphysema and Nonemphysema Groups

Group	*n*	Age	Pack-years	$FEV_{1.0}$%	DL_{CO}/VA	LAA	C/C	C/T	T/T	AF
Nonsmoker	94	31 ± 6	0	NE	NE	NE	68	22	4	0.160
Smoker	110	67 ± 9	64 ± 32	55 ± 17	3.6 ± 1.6	6.2 ± 5.1	75	32	3	0.173
Non-emphysema	65	66 ± 10	61 ± 31	60 ± 16	4.3 ± 1.2	2.5 ± 2.3	50	14	1	0.123
Emphysema	45	68 ± 8	68 ± 33	49 ± 17	2.6 ± 1.5	11.6 ± 2.7	25	18	2	0.244
P value		0.11	0.28	0.0006	<0.0001	<0.0001				0.02

Note. Values are expressed as mean ± SD. Pack-years, [packs per day × duration (years) of smoking]; $FEV_{1.0}$%, (forced expiratory volume in one second/forced vital capacity) × 100; DL_{CO}/VA, diffusing capacity of the lung for carbon monoxide per liter alveolar volume (ml/min/mmHg/l); LAA, low attenuation area score; C/C, subjects with −1562C/C genotype; C/T, those with C/T genotype; T/T, those with T/T genotype; AF, allele frequency of T allele; NE, not examined. *P* values between nonemphysema and emphysema groups are presented.

The smoker group consisted of clinical and subclinical COPD patients who visited the outpatient clinic of Keio University Hospital between 1998 and 2000. We excluded patients with giant bullae, pulmonary fibrosis, diffuse bronchiectasis, pulmonary vascular disease, bronchial asthma, or lung cancer compromising pulmonary function. Healthy volunteers were enrolled as the nonsmoker group. The average age of the smoker and nonsmoker groups was 67 ± 9 and 31 ± 6 years old, respectively (mean ± SD).

Clinical evaluation. Chest CT-scan and pulmonary function tests were performed in all subjects in the smoker group. Low attenuation area (LAA) was visually assessed according to the method reported previously (11). The total number of points (0–24) was used as the LAA score representing emphysematous changes. Evaluation of the LAA score was made by three pulmonologists in a blinded manner, and the mean score was used as a quantitative indicator of emphysematous change in each subject. Informed consent was obtained from each subject and the study protocol was approved by the Ethics Committee of Keio University Hospital.

Statistical analysis. Values are presented as mean ± SD. Allele frequencies were compared between groups by χ^2 test. Mean values of age, CC, $FEV_{1.0}$%, DL_{CO}/VA, and LAA score were compared between groups using unpaired Student's *t*-test. Multiple logistic regression analysis was performed to evaluate independent contribution of each factor to pulmonary emphysema. A *P* value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The smoker group was divided into two groups based on the LAA score on chest CT-scans; emphysema group ($n = 45$, LAA ≥ 8.0) and nonemphysema group ($n = 65$, LAA <8.0) (Table 1). This value of LAA has been reported as one of the criteria for the diagnosis of pulmonary emphysema (12). Although there was no difference in −1562T allele frequency between the smoker group and nonsmoker group, the frequency was obviously higher in the emphysema group than in the nonemphysema group ($\chi^2 = 5.48$, P = 0.02) in the absence of a significant difference in age and CC between the two groups (Table 1). There were differences in age and a proportion of genders between the smoker and nonsmoker groups, which may affect the T allele frequency in these groups. However, no difference was observed in mean age between subjects with and without the T allele in a total population ($n = 204$, 52 ± 21 vs 50 ± 19, $P = 0.61$). Neither was there difference in the T allele frequency between genders ($n = 204$, male: 0.163 vs female: 0.183, $P = 0.66$). The distributions of genotypes were consistent with Hardy–Weinberg equilibrium in the smoker and nonsmoker groups. To examine the relative importance of the independent contributions of CC, age, and genotype to the development of pulmonary emphysema, logistic regression analysis was performed. Having at least one −1562T allele was a significant risk factor for the development of pulmonary emphysema, while increase in CC or age did not correlate with the development of pulmonary emphysema after adjustment for the other factors (Table 2). When lung function parameters and LAA scores were compared between the genotypes, DL_{CO}/VA was lower in the C/T or T/T group than in the C/C group, while the LAA score was higher in the former than in the latter (Table 3). There was no significant difference in $FEV_{1.0}$% between the genotypes.

The present study suggests that the −1562C/T transition of the MMP-9 gene is associated with the development of pulmonary emphysema induced by cigarette

TABLE 2

Logistic Regression Analysis of Factors Related to Pulmonary Emphysema

	χ^2	*P*	Odds ratio	(95% CI)
CC (10 pack-years)	0.87	0.35	1.06	(0.94–1.20)
Age (10 years old)	1.52	0.22	1.32	(0.85–2.06)
Genotype (having a T allele)	5.35	0.02	2.69	(1.16–6.23)

Note. CC, lifelong cigarette consumption (pack-years). Odds ratio and 95% CI indicate effects of increase in 10 pack-years of CC, 10 years of age, or having at least one T allele on belonging to the emphysema group.

TABLE 3

Difference in D_{LCO}/VA and LAA Score between Genotypes

Genotype	*n*	Age	Pack-years	$FEV_{1.0}$%	D_{LCO}/VA	LAA
C/C	75	66 ± 9	65 ± 35	57 ± 18	3.8 ± 1.5	5.5 ± 5.0
C/T or T/T	35	68 ± 10	62 ± 25	52 ± 17	3.1 ± 1.6	7.7 ± 5.1
P value		0.47	0.65	0.25	0.02	0.03

Note. Values are expressed as mean ± SD. Pack-years, [packs per day × duration (years) of smoking]; $FEV_{1.0}$%, (forced expiratory volume in one second/forced vital capacity) × 100; D_{LCO}/VA, diffusing capacity of the lung for carbon monoxide per liter alveolar volume (ml/min/mmHg/l); LAA, low attenuation area score.

smoking. Interestingly, the allele frequency did not differ between the smoker group and nonsmoker group consisting of relatively younger subjects. These observations imply that nonsmokers in the younger generation with the T allele could develop pulmonary emphysema if they smoked substantially for a long time. A recent paper has suggested that women appear to be more sensitive to smoking than men for the development of airway obstruction (13). The smoker group in the present study mainly consisted of male patients, which reflected a general proportion of genders in COPD patients. Further investigations will be necessary to clarify the contribution of the MMP-9 gene polymorphism to the development of pulmonary emphysema in each gender. MMP-9 is produced by macrophages and neutrophils and degrades elastin as well as gelatin and collagen, and its expression is enhanced by various cytokines and growth factors (3–6). Since the inflammatory processes in COPD are characterized by the activation of alveolar macrophages and/or neutrophils, smokers with the T allele may develop pulmonary emphysema more rapidly than those with the C/C genotype. Zhang *et al.* demonstrated that the T allele had a higher promoter activity than the C allele by transient reporter gene expression assays, which appeared to be due to preferential binding of a putative transcription repressor protein to the C allelic promoter by DNA-protein interaction assays (10). The percentage of subjects with the T allele (32% in the smoker group) appears to be higher than that generally believed in epidemiological studies (10–20%). However, the phenotypes of COPD patients having each genotype corresponded reasonably to the difference in promoter activity of MMP-9 (Table 3) (10). Although there was no difference in $FEV_{1.0}$%, which is a representative parameter of overall airflow limitation, emphysematous changes assessed by the LAA score significantly differed between genotypes. This finding was confirmed by the fact that there was a significant difference in DL_{CO}/VA, which is known to well correlate with the extent of pulmonary emphysema among various parameters of pulmonary functions. These observations not only indicate that this polymorphism, in part, accounts for sensitivity to smoking, but also suggest that MMP-9 may be an important protease related to the pathogenesis of pulmonary emphysema. The −1562T allele is also known to be relatively common in Caucasians as well as in Japanese (10). Although multiple genetic factors, including the polymorphisms of substances involved in oxidant-antioxidant imbalance such as epoxide hydrolase (14), should be simultaneously considered to understand the entire picture of smoking sensitivity, the present study demonstrated that the polymorphism of MMP-9 could act as an intrinsic factor determining sensitivity to smoking in the development of pulmonary emphysema.

ACKNOWLEDGMENTS

The authors thank Fumihiro Yamasawa, M.D., Seitaro Fujishima, M.D., and Makiko Iwata, M.S. for their assistance.

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