

# Matrix Metalloproteinase-9 Promoter Polymorphism Associated with Upper Lung Dominant Emphysema

Isao Ito, Sonoko Nagai, Tomohiro Handa, Shigeo Muro, Toyohiro Hirai, Mitsuhiro Tsukino, and Michiaki Mishima

Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

**Rationale:** Matrix metalloproteinase 9 (MMP-9) has proteolytic activity against connective tissue proteins and appears to play an important role in the development of chronic obstructive pulmonary disease (COPD). The functional polymorphism of MMP-9 (C-1562T) is considered as one of the candidate genes in the susceptibility to COPD.

**Objectives:** To determine if MMP-9 (C-1562T) is related to the development of COPD in the Japanese population and whether it is associated with development of pulmonary emphysema assessed by high-resolution computed tomographic (HRCT) parameters.

**Methods:** MMP-9 (C-1562T) genotypes of 84 patients with COPD and 85 healthy smokers (control subjects) were determined by the restriction fragment length polymorphism method. We investigated the relationship between the genotypes using automatically analyzed HRCT parameters, such as percentage of low attenuation area (LAA%) and average computed tomography (CT) value density (Hounsfield units; mean CTv) in upper, middle, and lower lung fields in all patients with COPD.

**Measurements and Main Results:** There was no difference in polymorphism of MMP-9 (C-1562T) between patients with COPD and control subjects. In the HRCT study, patients with COPD with a T allele (C/T or T/T) showed larger LAA% (95% confidence interval of difference, 0.5–18.7;  $p = 0.04$ ), and smaller mean CTv (confidence interval,  $-34.3$  to  $-1.0$ ;  $p = 0.04$ ) in the upper lung compared with patients without T alleles (C/C). However, pulmonary function tests showed no difference between the two patient groups. Patients with a T allele showed a decrease in LAA% and an increase in mean CTv from upper to lower lung fields ( $p = 0.006$  and  $p = 0.002$ , respectively).

**Conclusions:** Polymorphism of MMP-9 (C-1562T) was associated with upper lung dominant emphysema in patients with COPD.

**Keywords:** chronic obstructive pulmonary disease; high-resolution computed tomography; low attenuation area %; mean CT value

Chronic obstructive pulmonary disease (COPD) is characterized by decreased expiratory flow rates. The most significant factor for developing COPD is cigarette smoking (1). However, it is estimated that only 15 to 20% of chronic smokers develop this disease, which is characterized by rapid decline of FEV<sub>1</sub> (2, 3). There are several epidemiologic studies that demonstrate familial clustering of the disease (4, 5). These facts suggest that genetic factors have a role in contributing to an individual's susceptibility to COPD. Of the candidate genes investigated in relation to COPD development, those coding for matrix metalloproteinases (MMPs) have attracted attention under the widely accepted

protease–antiprotease imbalance theory associated with the pathogenesis of this disease (6–12).

MMP-9, also called gelatinase B, has been proposed to play a role in the development of emphysema and is involved in the digestion of extracellular matrix components such as gelatin, collagens (IV, V, XI, XVII), and elastin (13). The human MMP-9 gene is located on chromosome 20q11.1–13.1, and MMP-9 is synthesized as a proenzyme with a molecular mass of 92 kD. Among several polymorphic changes reported in the regulatory region (14, 15), the C-1562T polymorphism increases the promoter activity of MMP-9 (16, 17). This polymorphism was shown to have a possible role in development of atherosclerotic diseases (16, 18, 19). In COPD, there are few studies investigating the relationship of the polymorphism to the development of the disease (20–22). Minematsu and colleagues evaluated the degree of emphysematous changes by a visual scoring system using conventional computed tomography (CT) in a Japanese population, and found that smokers with the T allele showed more severe emphysema than smokers without the T allele (20). Zhou and colleagues showed that C-1562T was more frequent in patients with COPD diagnosed by pulmonary function tests, compared with control subjects in a Chinese population (22). However, Joos and colleagues reported that fast decline of FEV<sub>1</sub> was not related to the polymorphism among white smokers (21). Thus, the role of the MMP-9 C-1562T polymorphism in association with COPD has been assessed by a variety of methods and the issue remains inconclusive.

To test the hypothesis that the MMP-9 C-1562T polymorphism has an important role in susceptibility to developing COPD, we first analyzed the polymorphisms in a sample of patients with COPD and healthy smokers (control subjects) from the Japanese population. Automatically calculated parameters, such as percentage of low attenuation area (LAA%) and mean CT value (mean CTv) in high-resolution CT (HRCT), have previously been shown to be useful in assessment of the emphysema-associated parenchymal injuries seen in patients with COPD (23–26). Second, using radiologic parameters assessed by HRCT, we examined patients with COPD for the correlation between the genotypes and phenotypes of emphysema.

## METHODS

### Subjects

The study comprised 84 patients with COPD and 85 healthy smokers. The subjects were included according to criteria established in previous studies (26, 27), with minor modifications. Briefly, COPD was diagnosed according to the Global Initiative for Obstructive Lung Disease (GOLD) guidelines (28), and no patient with  $\alpha_1$ -antitrypsin deficiency was included. This study was approved by the ethics committee of Kyoto University, and written, informed consent was obtained from all subjects.

### Genotyping

A standard phenol-chloroform method was used to extract DNA from blood. Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis were performed to detect a point

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Correspondence and requests for reprints should be addressed to Sonoko Nagai, M.D., Ph.D., Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Shogoin-kawaharacho 54, Sakyo-ku, Kyoto, 606-8507, Japan. E-mail: nagai@kuhp.kyoto-u.ac.jp

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TABLE 1. BASELINE CHARACTERISTICS OF STUDY SUBJECTS

	Men/Women	Age (yr)	Smoking History (pack-yr)	FVC (L)	FEV <sub>1</sub> (%pred)	FEV <sub>1</sub> /FVC (%)
Patients with COPD, n = 84	81/3	68.9 ± 7.5	58.1 ± 28.9	2.58 ± 0.80	44.9 ± 17.4	45.3 ± 9.8
Control smokers, n = 85	69/16	58.8 ± 12.8	41.4 ± 22.4	3.33 ± 1.11	88.5 ± 20.9	80.2 ± 7.9

Definition of abbreviation: COPD = chronic obstructive pulmonary disease. Values are mean ± SD.

mutation at the promoter of C-1562T of the MMP-9 gene as described by Zhang and colleagues (16). PCR was performed with a thermal cycler (DNA Thermal Cycler; Perkin Elmer Cetus, Norwalk, CT) with the following cycling parameters: 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s, for 35 cycles. The PCR products were digested with restriction enzyme *Bbu* I (Takara Bio, Otsu, Japan) at 37°C for 4 h, resolved on 3% agarose gels, stained with ethidium bromide (Invitrogen, Carlsbad, CA), and observed under ultraviolet light.

### Measurement of Chest HRCT Parameters in Patients with COPD

All CT images were taken on subjects in the supine position with an HRCT scanner (X-Vigor; Toshiba, Tokyo, Japan), with 2-mm collimation according to the previously reported protocol (23–26). Three images were used for analysis for each patient: upper lung field at 1 cm above the upper margin of the aortic arch, middle lung at 1 cm below the carina, and lower lung at 3 cm above the diaphragm (23, 25). Contiguous pixels with less than -960 Hounsfield units (HU) were defined as LAA and the percentage ratio of LAA in both lung fields on each images (LAA%) was calculated automatically (23, 25): LAA-U%, at the upper lung field; LAA-M%, at the middle; LAA-L%, at the lower; and LAA-T%, for the average of the three images. LAA-T% was calculated as  $\Sigma(\text{LAA-X}\% \times \text{no. of lung pixels in X}) / (\text{total no. of pixels of lung fields in three images})$ . Mean CTv-U, mean CTv-M, and mean CTv-L were calculated as the average of the CT values in HU of all pixels in both lung fields at the upper, middle, and lower lung fields, respectively. Mean CTv-T was defined described for LAA-T%.

### Statistical Analysis

To test frequencies of the MMP-9 C-1562T polymorphism,  $\chi^2$  tests for the equality of two population probabilities were performed. A two-tailed *t* test was used to test the difference of averages in the pulmonary function tests and the HRCT parameters between T(+) subjects (with C/C genotype) and T(-) subjects (with C/T or T/T genotype). Analysis of variance (ANOVA) with repeated measures was used to explore the effect of image levels on HRCT parameters separately for each patient group. For multiple comparisons, a *post hoc* test with Bonferroni/Dunn's method was used. StatView version 5.0 (SAS Institute, Inc., Cary, NC) was used for the calculations. *p* values less than 0.05 were considered to be significant.

### RESULTS

The baseline characteristics and the results of the pulmonary function tests for the 84 patients with COPD and 85 control subjects (healthy smokers) are presented in Table 1. In the initial evaluation of patients with COPD, two patients were classified as having stage I disease, 33 as having stage II, 30 as having stage III, and 19 as having stage IV disease, according to the revised GOLD criteria (28).

The genotype frequencies in each group are shown in Table 2. There were no significant differences in frequencies of alleles and genotypes between patients with COPD and control subjects. Because the T allele has a codominant effect on plasma MMP-9 level (17), we divided patients with COPD and control subjects into two groups for comparison of clinical parameters between genotypes: T(-) subjects (with C/C genotype) and T(+) subjects (with C/T or T/T genotype). Pulmonary functions were not significantly different between the T(+) and T(-) groups among

either patients with COPD (Table 3) or control subjects (data not shown).

Chest HRCT examination was performed to obtain HRCT parameters in all patients with COPD. Results of LAA% and mean CTv calculated in each genotype group are shown in Figures 1 and 2. Although LAA-M%, LAA-L%, and LAA-T% between T(-) and T(+) patients were not significantly different (*p* = 0.23, 0.45, and 0.15, respectively), LAA-U% was notably higher in T(+) patients than in T(-) patients (95% confidence interval [CI], 0.5–18.7; *p* = 0.04; Figure 1). In the measurement of mean CTv, mean CTv-U was considerably lower in T(+) patients than in T(-) patients (95% CI, -34.3 to -1.0; *p* = 0.04), whereas mean CTv-M, mean CTv-L, and mean CTv-T were not significantly different (*p* = 0.28, 0.70, and 0.20, respectively; Figure 2). In comparisons among the three image positions in each patient group by ANOVA with repeated measures, LAA% in T(+) patients significantly decreased as the positions of the images moved from upper to lower (*p* = 0.006), whereas LAA% in T(-) patients did not change (*p* = 0.53; Figure 1). Mean CTv in T(+) patients significantly increased as the positions moved from upper to lower (*p* = 0.002), whereas mean CTv in T(-) patients did not change (*p* = 0.32; Figure 2). There was no significant difference between patients with T/T genotype and patients with C/T genotype in any HRCT parameters (data not shown).

### DISCUSSION

This study demonstrated that the T allele at the MMP-9 C-1562T polymorphism was significantly associated with upper lung dominant emphysema in patients with COPD, although the polymorphism was not associated with the development of COPD in the Japanese population. Previous studies reporting on the association of this polymorphism with the risk of developing COPD have shown variable results (20–22). Our results corroborate those of Minematsu and colleagues (20), showing that the T allele has some role in the progression of emphysema in lung parenchyma, at least in the Japanese population. We reported that T(+) patients are linked with upper lung dominant emphysema but not with further deterioration of lung function, compared with T(-) patients. In addition, our data show this polymorphism to have

TABLE 2. ALLELIC AND GENOTYPIC FREQUENCIES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND HEALTHY CONTROL SMOKERS

	COPD (n = 84)	Control (n = 85)	<i>p</i> Value
Allele			
C	145 (86%)	144 (85%)	0.79
T	23 (14%)	26 (15%)	
Genotype			
CC	63 (75%)	60 (71%)	0.61
CT	19 (23%)	24 (28%)	
TT	2 (2%)	1 (1%)	

For definition of abbreviation, see Table 1.

**TABLE 3. COMPARISON OF PULMONARY FUNCTIONS BETWEEN T(-) PATIENTS AND T(+) PATIENTS, BOTH WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

	Men/Women	Age (yr)	Smoking History (pack-yr)	FVC (L)	FEV <sub>1</sub> (%pred)	FEV <sub>1</sub> /FVC (%)	D <sub>Lco</sub> /VA*
T(-) patients, n = 63	60/3	68.2 ± 7.6	59.2 ± 29.6	2.55 ± 0.82	44.4 ± 17.4	45.3 ± 9.7	3.50 ± 1.41
T(+) patients, n = 21	21/0	70.7 ± 6.7	54.7 ± 29.5	2.67 ± 0.70	46.3 ± 16.9	45.1 ± 10.0	2.96 ± 1.11
p Value		0.19	0.56	0.54	0.67	0.92	0.14

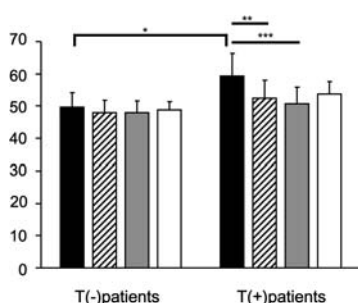
Definition of abbreviation: D<sub>Lco</sub>/VA = diffusing capacity of the lung for carbon monoxide per unit of alveolar volume.

Values are mean ± SD.

\* Nos. of patients measured are 51 in T(-) group and 19 in T(+) group.

no association with an increased risk of COPD development, determined by the decreased value of FEV<sub>1</sub>/FVC and not by radiographic evaluation, and has some accordance with the result by Joos and colleagues, who evaluated the risk by fast rate of FEV<sub>1</sub> decline (21). We believe that discrepancies in the role of the T allele between the HRCT findings and pulmonary function are due to emphysematous change in the upper lung having less influence on the decrease of FEV<sub>1</sub> than the influence of emphysema in the lower lung (29).

Two major types of parenchymal destruction in pulmonary emphysema are differentiated in the general population, and are described as centrilobular emphysema and panlobular emphysema (26, 30–32). Centrilobular emphysema begins in and around the respiratory bronchioles, resulting in dilatation and destruction of the lobule center. A predilection of centrilobular emphysema for the upper lung, rather than lower lung, has been noted. Panlobular emphysema, also referred to as panacinar emphysema, affects the acinus of the entire secondary lobule. This latter type of emphysema extends diffusely throughout the lung, with some preferential involvement in the lower lung. In addition to the histopathologic differences, differences in pathophysiology, such as elasticity (33) and airway reactivity (34), between the two types of emphysema have been noted in smokers. Because the phenotypes for centrilobular and panlobular emphysema are different, there could also be differences between the progressions of the two types of emphysema. Because we did not investigate lung pathology of patients with COPD, we were not able to differentiate the two types of emphysema in our subjects. Considering that centrilobular emphysema is the dominant type linked to cigarette smoking, it does not seem adequate to interpret the presence or absence of the T allele as simply corresponding to the centrilobular or panlobular type of emphysema.



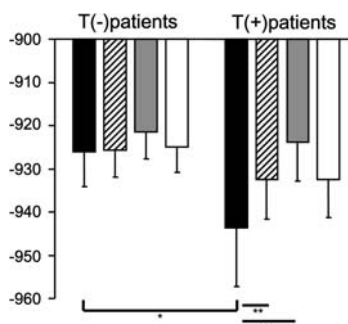
**Figure 1.** Percentage of low attenuation area (LAA%) in T(+) and T(-) patients. Black bars denote LAA% in the upper lung (LAA-U%), hatched bars denote LAA% in the middle lung (LAA-M%), gray bars denote LAA% in the lower lung (LAA-L%), and white bars denote LAA% for the average of the three lung fields (LAA-T%). Error bars depict the 95% confidence interval.

\*Bracket shows comparison and significant difference in LAA-U% between T(+) and T(-) patients ( $p = 0.04$ ). By analysis of variance (ANOVA) with repeated measures, there was a significant effect of slice position of high-resolution computed tomography (HRCT) among T(+) patients ( $p = 0.006$ ), but not in T(-) patients ( $p = 0.53$ ). \*\* $p = 0.01$ ; \*\*\* $p < 0.01$ .

Few attempts have been made to investigate a link between genetic factors and emphysematous changes in HRCT (26, 35). A correlation study between HRCT and the pathologic findings used to discriminate between the two types of emphysema is needed to develop a protocol for using HRCT to discriminate between the two types. It would then be possible to determine the mechanism whereby the MMP-9 promoter C-1562T polymorphism contributes to the development of upper lung dominant emphysema. Most patients with COPD in our study had stage II–IV disease, and only a very few had stage 0–I. It would extend our results to use HRCT to investigate patients with 0–I stage disease to determine whether this polymorphism contributes to early development of upper lung dominant emphysema; this is difficult to detect by pulmonary function tests.

The primary mechanism of lung parenchymal destruction is believed to be an imbalance between endogenous proteinases and antiproteinases. The significant biological function of MMP-9, which is related to destruction of lung parenchyma, is considered to be due to its proteolytic effect on substrates such as extracellular matrix proteins and antiproteinases. Although the two types of emphysema may show similar distributions within the lung during advanced disease stages, and may sometimes coexist (30, 36), there is believed to be some difference in the way proteinase–antiproteinase imbalance affects the progression of emphysema (37).

MMP-9 is expressed by many kinds of inflammatory cells, such as alveolar macrophages, neutrophils, and eosinophils (13). Among them, alveolar macrophages are likely to have an important pathogenic role in emphysema (38, 39). This protein is also produced by structural cells in the lung, such as bronchial epithelial cells, alveolar type II cells, and smooth muscle cells, in appropriate conditions (13). The role of MMP-9 has been



**Figure 2.** Mean computed tomographic (CT) value (mean CTv) in T(+) and T(-) patients. Black bars denote mean CTv in the upper lung (mean CTv-U), hatched bars denote mean CTv in the middle lung (mean CTv-M), gray bars denote mean CTv in the lower lung (mean CTv-L), and white bars denote mean CTv for the average of the three lung fields (mean CTv-T). Error bars depict the 95% confidence interval.

\*Bracket shows comparison and significant difference in mean CTv-U between T(+) and T(-) patients ( $p = 0.04$ ). By ANOVA with repeated measures, there was a significant effect of slice position of HRCT among T(+) patients ( $p = 0.002$ ), but not in T(-) patients ( $p = 0.32$ ). \*\* $p < 0.05$ ; \*\*\* $p < 0.001$ .

shown to be important in the development of emphysema in animal (40–44) and human (6, 7, 9–12, 39, 45, 46) studies. Histopathologically, MMP-1, MMP-2, MMP-8, and MMP-9 showed increased expression in the lungs of patients with COPD (9). However, mRNA of MMP-1, not MMP-9 or MMP-12, was expressed more in the lungs of patients with severe emphysema than in those of normal subjects (47). In murine models of emphysema induced by chronic cigarette smoke, Churg and colleagues emphasized the relevance of MMP-12 in relation to tumor necrosis factor  $\alpha$  (48). Thus, there is debate that other MMPs may also play important roles in the destruction of alveolar walls.

Why some patients with COPD present upper lung dominant emphysema and how the T allele of the MMP-9 promoter is related to this phenotype is still unknown and requires further study. Considering that C-1562T polymorphism increases the promoter activity (16, 17), it could be inferred that MMP-9 activity plays a more important role in the destruction of alveolar walls in the upper lung than in the lower lung. Because MMP-9 alone does not digest collagen type I/III, which is the primary element of alveolar walls sustaining the mechanical force of breathing (49, 50), other enzymes mentioned above may also be preferentially expressed in the upper lung, and collaborate with MMP-9 in the digestion of the extracellular matrix. After proteolytic digestion of the extracellular matrix, with resultant weakened remodeling of the collagen network, mechanical force plays an important role in destroying alveolar walls (49–51). Distribution of the mechanical stress is larger in the upper lung due to the overall weight of the lung (52), and could contribute to the preferential progression of emphysema in the upper lung of certain patients. Our data suggest that, in a Japanese population with COPD, the C-1562T polymorphism could affect the distribution and the progression of emphysema, not the susceptibility to COPD (decline of FEV<sub>1</sub>).

In conclusion, we have shown that C-1562T polymorphism in the MMP-9 promoter was not associated with development of COPD diagnosed by pulmonary function tests. However, the T allele was significantly associated with the development of upper lung dominant emphysema in patients with COPD. The role of MMP-9 promoter genotypes in the progression of emphysema remains to be elucidated.

**Conflict of Interest Statement:** None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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## References

- Snider GL. Chronic obstructive pulmonary disease: risk factors, pathophysiology and pathogenesis. *Annu Rev Med* 1989;40:411–429.
- Hoshino Y, Nagai S, Koyama H, Okuda K, Nishimura K, Miki H, Hamada K, Izumi T. Airflow limitation in Japanese smokers: significance of serum neutrophil elastase/ $\alpha$ 1-proteinase inhibitor ratio and FEV<sub>1</sub> (% pred.) adjusted by pack-years. *Respiration (Herrlisheim)* 2000; 67:372–377.
- Fletcher C, Peto R. The natural history of chronic airflow obstruction. *BMJ* 1977;1:1645–1648.
- Khoury MJ, Beaty TH, Newill CA, Bryant S, Cohen BH. Genetic-environmental interactions in chronic airways obstruction. *Int J Epidemiol* 1986;15:65–72.
- Kueppers F, Miller RD, Gordon H, Hepper NG, Offord K. Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am J Med* 1977;63:336–342.
- Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Konttinen YT. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. *Lab Invest* 1998;78:1077–1087.
- Betsuyaku T, Nishimura M, Takeyabu K, Tanino M, Venge P, Xu S, Kawakami Y. Neutrophil granule proteins in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Am J Respir Crit Care Med* 1999;159:1985–1991.
- Lim S, Roche N, Oliver BG, Mattos W, Barnes PJ, Chung KF. Balance of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 from alveolar macrophages in cigarette smokers: regulation by interleukin-10. *Am J Respir Crit Care Med* 2000;162:1355–1360.
- Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M. Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. *Chest* 2000;117:684–694.
- Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2002;26:602–609.
- Mao JT, Tashkin DP, Belloni PN, Baileyhealy I, Baratelli F, Roth MD. All-trans retinoic acid modulates the balance of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in patients with emphysema. *Chest* 2003;124:1724–1732.
- Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med* 2003;97:634–639.
- Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol* 2003;28:12–24.
- Zhang B, Henney A, Eriksson P, Hamsten A, Watkins H, Ye S. Genetic variation at the matrix metalloproteinase-9 locus on chromosome 20q12.2–13.1. *Hum Genet* 1999;105:418–423.
- Hirakawa S, Lange EM, Colicigno CJ, Freedman BI, Rich SS, Bowden DW. Evaluation of genetic variation and association in the matrix metalloproteinase 9 (MMP9) gene in ESRD patients. *Am J Kidney Dis* 2003;42:133–142.
- Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, Luc G, Cambien F, Hamsten A, *et al.* Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 1999;99:1788–1794.
- Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G, Meyer J, Cambien F, Tiret L; AtheroGene Investigators. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 2003;107:1579–1585.
- Jones GT, Phillips VL, Harris EL, Rossaak JI, van Rij AM. Functional matrix metalloproteinase-9 polymorphism (C-1562T) associated with abdominal aortic aneurysm. *J Vasc Surg* 2003;38:1363–1367.
- Morgan AR, Zhang B, Tapper W, Collins A, Ye S. Haplotypic analysis of the MMP-9 gene in relation to coronary artery disease. *J Mol Med* 2003;81:321–326.
- Minematsu N, Nakamura H, Tateno H, Nakajima T, Yamaguchi K. Genetic polymorphism in matrix metalloproteinase-9 and pulmonary emphysema. *Biochem Biophys Res Commun* 2001;289:116–119.
- Joos L, He JQ, Shepherdson MB, Connett JE, Anthonisen NR, Pare PD, Sandford AJ. The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet* 2002;11:569–576.
- Zhou M, Huang SG, Wan HY, Li B, Deng WW, Li M. Genetic polymorphism in matrix metalloproteinase-9 and the susceptibility to chronic obstructive pulmonary disease in Han population of south China. *Chin Med J (Engl)* 2004;117:1481–1484.
- Sakai N, Mishima M, Nishimura K, Itoh H, Kuno K. An automated method to assess the distribution of low attenuation areas on chest CT scans in chronic pulmonary emphysema patients. *Chest* 1994;106: 1319–1325.
- Mishima M, Hirai T, Itoh H, Nakano Y, Sakai H, Muro S, Nishimura K, Oku Y, Chin K, Ohi M, *et al.* Complexity of terminal airspace geometry assessed by lung computed tomography in normal subjects and patients with chronic obstructive pulmonary disease. *Proc Natl Acad Sci USA* 1999;96:8829–8834.
- Mishima M, Oku Y, Kawakami K, Sakai N, Fukui M, Hirai T, Chin K, Ohi M, Nishimura K, Itoh H, *et al.* Quantitative assessment of the spatial distribution of low attenuation areas on X-ray CT using texture analysis in patients with chronic pulmonary emphysema. *Front Med Biol Eng* 1997;8:19–34.
- Ito I, Nagai S, Hoshino Y, Muro S, Hirai T, Tsukino M, Mishima M. Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. *Chest* 2004;125:63–70.
- Nakano Y, Muro S, Sakai H, Hirai T, Chin K, Tsukino M, Nishimura K, Itoh H, Pare PD, Hogg JC, *et al.* Computed tomographic measurements

- of airway dimensions and emphysema in smokers: correlation with lung function. *Am J Respir Crit Care Med* 2000;162:1102–1108.
28. GOLD. Global initiative for chronic obstructive lung disease. Workshop report: global strategy for the diagnosis, management and prevention of COPD. Bethesda, MD: National Institutes for Health; 2004; [accessed 8 Sept 2004]. Available from: <http://www.goldcopd.com>.
  29. Saitoh T, Koba H, Shijubo N, Tanaka H, Sugaya F. Lobar distribution of emphysema in computed tomographic densitometric analysis. *Invest Radiol* 2000;35:235–243.
  30. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004;364:709–721.
  31. Anderson AE Jr, Foraker AG. Centrilobular emphysema and panlobular emphysema: two different diseases. *Thorax* 1973;28:547–550.
  32. Thurlbeck WM. The incidence of pulmonary emphysema, with observations on the relative incidence and spatial distribution of various types of emphysema. *Am Rev Respir Dis* 1963;87:206–215.
  33. Kim WD, Eidelman DH, Izquierdo JL, Ghezzi H, Saetta MP, Cosio MG. Centrilobular and panlobular emphysema in smokers: two distinct morphologic and functional entities. *Am Rev Respir Dis* 1991;144:1385–1390.
  34. Finkelstein R, Ma HD, Ghezzi H, Whittaker K, Fraser RS, Cosio MG. Morphometry of small airways in smokers and its relationship to emphysema type and hyper-responsiveness. *Am J Respir Crit Care Med* 1995;152:267–276.
  35. Sakao S, Tatsumi K, Igari H, Watanabe R, Shino Y, Shirasawa H, Kuriyama T. Association of tumor necrosis factor- $\alpha$  gene promoter polymorphism with low attenuation areas on high-resolution CT in patients with COPD. *Chest* 2002;122:416–420.
  36. Mitchell RS, Silvers GW, Goodman N, Dart G, Maisel JC. Are centrilobular emphysema and panlobular emphysema two different diseases? *Hum Pathol* 1970;1:433–441.
  37. Cockcroft DW, Horne SL. Localization of emphysema within the lung: an hypothesis based upon ventilation/perfusion relationships. *Chest* 1982;82:483–487.
  38. Finkelstein R, Fraser RS, Ghezzi H, Cosio MG. Alveolar inflammation and its relation to emphysema in smokers. *Am J Respir Crit Care Med* 1995;152:1666–1672.
  39. Finlay GA, O'Driscoll LR, Russell KJ, D'Arcy EM, Masterson JB, Fitzgerald MX, O'Connor CM. Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. *Am J Respir Crit Care Med* 1997;156:240–247.
  40. Wang Z, Zheng T, Zhu Z, Homer RJ, Riese RJ, Chapman HA Jr, Shapiro SD, Elias JA. Interferon gamma induction of pulmonary emphysema in the adult murine lung. *J Exp Med* 2000;192:1587–1600.
  41. Wert SE, Yoshida M, LeVine AM, Ikegami M, Jones T, Ross GF, Fisher JH, Korfhagen TR, Whitsett JA. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. *Proc Natl Acad Sci USA* 2000;97:5972–5977.
  42. Selman M, Cisneros-Lira J, Gaxiola M, Ramirez R, Kudlacz EM, Mitchell PG, Pardo A. Matrix metalloproteinases inhibition attenuates tobacco smoke-induced emphysema in Guinea pigs. *Chest* 2003;123:1633–1641.
  43. Choe KH, Taraseviciene-Stewart L, Scerbavicius R, Gera L, Tuder RM, Voelkel NF. Methylprednisolone causes matrix metalloproteinase-dependent emphysema in adult rats. *Am J Respir Crit Care Med* 2003;167:1516–1521.
  44. Lee JH, Lee DS, Kim EK, Choe KH, Oh YM, Shim TS, Kim SE, Lee YS, Lee SD. Simvastatin inhibits cigarette smoking-induced emphysema and pulmonary hypertension in rat lungs. *Am J Respir Crit Care Med* (In press)
  45. Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, Fitzgerald M, Barnes PJ. Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L867–L873.
  46. Montano M, Becceril C, Ruiz V, Ramos C, Sansores RH, Gonzalez-Avila G. Matrix metalloproteinases activity in COPD associated with wood smoke. *Chest* 2004;125:466–472.
  47. Imai K, Dalal SS, Chen ES, Downey R, Schulman LL, Ginsburg M, D'Armiento J. Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. *Am J Respir Crit Care Med* 2001;163:786–791.
  48. Churg A, Wang RD, Tai H, Wang X, Xie C, Dai J, Shapiro SD, Wright JL. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor- $\alpha$  release. *Am J Respir Crit Care Med* 2003;167:1083–1089.
  49. Suki B, Lutchen KR, Ingenito EP. On the progressive nature of emphysema: roles of proteases, inflammation, and mechanical forces. *Am J Respir Crit Care Med* 2003;168:516–521.
  50. Suki B, Ito S, Stamenovic D, Lutchen KR, Ingenito EP. Biomechanics of the lung parenchyma: critical roles of collagen and mechanical forces. *J Appl Physiol* 2005;98:1892–1899.
  51. Kononov S, Brewer K, Sakai H, Cavalcante FS, Sabayanagam CR, Ingenito EP, Suki B. Roles of mechanical forces and collagen failure in the development of elastase-induced emphysema. *Am J Respir Crit Care Med* 2001;164:1920–1926.
  52. West JB. Distribution of mechanical stress in the lung, a possible factor in localization of pulmonary disease. *Lancet* 1971;1:839–841.