Respiratory Medicine (2005) **99**, 107–110

respiratoryMEDICINE

Association analysis of tissue inhibitor of metalloproteinase2 gene polymorphisms with COPD in Egyptians

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Received 13 November 2003; accepted 28 May 2004

KEYWORDS
TIMP2;
COPD;
Polymorphism;
Egyptian

Summary Proteinase/antiproteinase imbalance is recognized to play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). A relative increase in the activities of matrix metalloproteinases might be caused by mutations of tissue inhibitor of metalloproteinase2 (TIMP2). Recently, two polymorphisms of the TIMP2 gene, +853 G/A and −418 G/C (+551 and −720 from the translation initiation site), have been shown to be associated with the development of COPD in the Japanese population. In this study, a case-control association analysis for these polymorphisms was conducted in the Egyptian population using 106 COPD patients and 72 healthy controls. The genotype frequency of +853 G/A was significantly different between the patient and the control groups ($P = 0.029$), although no significant difference was detected in the allele frequency between the two groups. These results suggest that the +853 G/A polymorphism of the TIMP2 gene might be associated with COPD across ethnicities. In contrast, neither the distributions of genotype nor allele frequencies of −418 G/C were significantly different between the two groups, raising the possibility that a combination of different genetic factors contributes to the development of COPD in different ethnic groups.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by slowly progressive airflow limitation that is largely irreversible.[1] The airflow limitation is caused by a mixture of small airway disease and parenchymal destruction. The etiology of COPD is multifactorial. An interaction between environmental and genetic factors has been recognized to be associated with the development of COPD.[2] Cigarette smoking is the most important risk factor. However, genetic factors are believed to play an important role in the susceptibility to COPD in smokers.[3] To date, more than 20 polymorphisms of candidate genes have been reported

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doi:10.1016/j.rmed.2004.05.017

to have an association with COPD.[4] Several of these genes are involved in proteinase/antiproteinase imbalance.[5–7] Proteinase/antiproteinase imbalance is the most widely accepted theory for the development of COPD. Increased activities of proteinases compared to antiproteinases might result in epithelial damage as well as parenchemal destruction.

We have shown previously that two polymorphisms of the tissue inhibitor of metalloproteinase2 (TIMP2) gene, +853 G/A and −418 G/C (+551 and −720 from the translation initiation site), have a significant association with the development of COPD in the Japanese population.[7] TIMP2 inhibits matrix metalloproteinases (MMPs). Therefore, these polymorphisms might down-regulate TIMP2 activity and increase the activities of MMPs, leading to proteinase/antiproteinase imbalance and degradation of the lung matrix. In this study, we investigated the relationship between these polymorphisms and COPD in the Egyptian population. Since genetic heterogeneity among different ethnic groups could have different effects on multifactorial complex diseases, it is important to confirm associations of polymorphisms with diseases in various populations.

Materials and methods

Subjects and DNA samples

All subjects studied were Egyptian chronic heavy smokers recruited from the department of chest diseases and tuberculosis at Cairo university hospital and affiliated hospitals: 106 COPD patients and 72 age-matched healthy controls. COPD was diagnosed based on past history, physical examination and spirometric data; forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio of <70%, according to the Global Initiative for COPD criteria.[1] Subjects with other significant respiratory diseases such as bronchial asthma, bronchiectasis and pulmonary tuberculosis were not included. Genomic DNA samples were extracted from whole blood using a QIAGEN DNA blood kit (QIAGEN, Hilden, Germany). Written informed consent was obtained from all the subjects and the study was approved by the ethics committees of the hospitals involved.

Genotyping of TIMP2 polymorphisms

Two SNPs, +853 G/A and −418 G/C, were genotyped using TaqMan allelic discrimination technique.[8] The nucleotide positions in this study are given relative to the transcription start site. A pair of primers flanking the SNP and a pair of oligonucleotide probes, one homologous to the mutant type labeled with VIC and the other homologous to the wild type labeled with 6-carboxyfluorescein (FAM), were designed and synthesized by Applied Biosystems (Foster City, CA) (Table 1). Each PCR included 20 ng of genomic DNA, 900 nM of each primer, 200 nM of each probe, and 1x TaqMan Universal PCR Master Mix (Applied Biosystems) in a volume of 25 μl. PCR cycling conditions in the ABI PRISM 7000 (Applied Biosystems) were as follows: 50°C for 2 min; 95°C for 10 min; followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The different alleles were discriminated according to the fluorescence intensity of FAM and VIC.

Table 1 Primers and probes used for TaqMan allelic discrimination.

Target SNP	Primers and probes
+853 G/A	
Primer:	
Forward	5′-CCCTCCTCGGCAGTGTGT-3′
Reverse	5′-CTGCAATGAGATATTCCTTCTTTCC-3′
Probe:	
	5′-[VIC]ACGTCCAGCGAGAC[MGB]-3′
	5′-[FAM]ACGTCCAGTGAGACC[MGB]-3′
−418 G/C	
Primer:	
Forward	5′-AAAGGGATCCTGTCAGTTTCTCAA-3′
Reverse	5′-TTTCCCCTTCAGCTCGACTCT-3′
Probe:	
	5′-[VIC]CCGAGGCTGGGCT[MGB]-3′
	5′-[FAM]ACGACGCTGGGCT[MGB]-3′

FAM, 6-carboxyfluorescein; MGB, minor groove binder.

Statistical analysis

All clinical data are presented as the mean ± SEM. Differences in clinical data between the COPD patients and the control subjects were checked with the two-sided Student's *t*-test. Hardy–Weinberg equilibrium was assessed using a goodness-of-fit χ^2 test for biallelic markers. Fisher's exact test was used to analyze the distribution of genotype and allele frequency. Statistical significance was defined as $P<0.05$. Since the two SNPs had been reported to be associated with COPD in the Japanese population,[7] we did not adopt the Bonferroni's correction for multiple comparisons.

Linkage disequilibrium between the two SNPs was calculated with the software SNPAlyze program (Dynacom, Mobara, Japan).

Results

One hundred and six COPD patients and 72 controls were studied (age, patient group: 62.5±0.9 years, control group: 59.0±1.0 years). All were males. Brinkman's index (the number of cigarettes/day-× the number of years) was 1050.0±60.3 for the patient group and 990.9±69.6 for the control group. No significant differences between the two groups were detected in age and smoking history. The spirometric data indicated that the patients suffered from severe airflow limitation (FEV_1/FVC, 44.3±1.2%), while data for the controls were within normal (FEV_1/FVC, 78.3±1.0%). The *P*-value was less than 0.001.

Table 2 shows the genotype and allele frequencies at position +853 and −418 both in the patients and in the controls. The genotype frequencies for both groups were consistent with Hardy–Weinberg equilibrium. The distribution of +853 G/A genotype frequencies was significantly different between the patients and the controls ($P = 0.029$). However the distribution of allele frequencies at +853 was not significant ($P = 0.206$). As for the −418 G/C polymorphism, neither genotype nor allele frequencies were significantly different between the two groups. There was no linkage disequilibrium between +853 G/A and −418 G/C.

Discussion

Genetic studies of complex diseases have been demonstrating conflicting results where some studies are showing positive association of a variant with a disease but others are showing no association. This might be due to the ethnic differences among the studies but it also might be due to the difference in the phenotype within the complex disease. In this study, we tried to replicate the association of two polymorphisms of the TIMP2 gene with COPD in the Egyptian population, which had been reported in the Japanese population.[7] In order to eliminate a possible effect of phenotype difference, we applied in this study the same recruitment criteria as that in the previous study.

The distribution of the +853 G/A genotype was significantly different between the COPD patients and the controls also in the Egyptian population. The consistent genetic association of this SNP with COPD across the different ethnicities supports the possibility of its involvement in the development of the disease. Although +853 G/A is a synonymous nucleotide substitution, this SNP can down-regulate the TIMP2 activity by influencing the secondary structure of the mRNA which inhibits ribosomal binding and/or decreases the mRNA stability.[9,10] It is also possible that this SNP is just in linkage disequilibrium with a yet to be identified—original causal polymorphism. There are numerous registered polymorphisms within TIMP2 gene and in its nearby genes, and any one of them might be functional and in linkage disequilibrium with +853 G/A.

As to the polymorphism at the −418 locus, the results of the present study were not consistent with those in the Japanese population. As the minor allele frequency at the −418 locus in Egyptians was 1.1% while that in Japanese was 17.2%,[7] it is possible that the frequency was too low in Egyptians to show a significant difference between the patient and the control groups of this number. It is also possible that the SNP at −418 is not involved in COPD pathogenesis in the Egyptian population. Even if its association with COPD was

Table 2 Genotype and allele frequencies of the TIMP2 gene polymorphisms.

	Genotypes *n* (%)			Alleles *n* (%)	
+853:	G/G	G/A	A/A	G	A
COPD	78 (73.6)	23 (21.7)	5 (4.7)	179 (84.4)	33 (15.6)
Controls	43 (59.7)	28 (38.9)	1 (1.4)	114 (79.2)	30 (20.8)
P-value	0.029			0.206	
−418:	G/G	G/C	C/C	G	C
COPD	104 (98.1)	2 (1.9)	0 (0)	210 (99.1)	2 (0.9)
Controls	70 (97.2)	2 (2.8)	0 (0)	142 (98.6)	2 (1.4)
P-value	NS			NS	

detected in Egyptians, the very low incidence of the SNP would indicate its minimal role in the pathogenesis. The incidence of polymorphisms can vary markedly between different ethnic groups.[11] Since the susceptibility to COPD is considered to be influenced by multiple genetic causes and genotype-by-environment interactions,[2] it is possible that different genetic variants in different ethnic groups cause the same COPD phenotype. Furthermore, since COPD is a heterogeneous disease caused by a mixture of small airway disease and parenchymal destruction, there is some possibility that the component of the Egyptian COPD group was different from that of the Japanese COPD group. These hypotheses might explain the discrepancy between studies showing a polymorphism to be associated with COPD in one population but not in others.[12–14]

In order to clarify the relationship between the two polymorphisms in this study and the development of COPD, further case-control studies in other ethnic groups are required. Additionally, functional characterization of these polymorphisms for TIMP2 might shed light on this point.

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