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

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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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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.