Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Matrix metalloproteinase gene polymorphisms: lack of association with chronic obstructive pulmonary disease in a Brazilian population

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Genet. Mol. Res. 8 (3): 1028-1034 (2009)
Received February 4, 2009
Accepted June 15, 2009
Published August 25, 2009

ABSTRACT. There are many candidate genes for chronic obstructive pulmonary disease (COPD). One such candidate is the group of genes that code for matrix metalloproteinases (MMPs), which play an essential role in tissue remodeling and repair associated with COPD. We tested the hypothesis that polymorphic variation in MMP genes influences the risk of developing COPD by examining functional polymorphisms in the promoters of MMP-3, MMP-9 and MMP-12 genes in 111 COPD patients and 101 controls. The -1171 5A/6A MMP-3, -1562 C/T MMP-9 and -82 A/G MMP-12 polymorphisms were analyzed by polymerase chain reaction, followed by restriction digestion. No significant differences were observed in allele and genotype frequencies between COPD patients and controls. Haplotype analysis also did not reveal differences between COPD patients and controls. We found that MMP polymorphisms had no

significant impact on the risk of developing COPD in this Brazilian sample.

Key words: Matrix metalloproteinases; Case-control studies; Single nucleotide polymorphism; Genetic predisposition to disease; Chronic obstructive pulmonary disease

INTRODUCTION

The increasing prevalence of chronic obstructive pulmonary disease (COPD) is an important public health concern. The disease is characterized by the progressive development of airflow limitation that is not fully reversible. Tobacco smoking represents the most significant environmental risk factor for the development of COPD. However, only a few smokers develop COPD, and the reason is still unknown (Siafakas and Tzortzaki, 2002).

Recently, research has focused on genetic variants of proteins that contribute to an individual's susceptibility to COPD. In a complex disease such as COPD, there are likely to be many genes contributing to the overall phenotype. 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 (Belvisi and Bottomley, 2003; Demedts et al., 2005; Gueders et al., 2006). Smokers with some specific MMP genotypes may be at increased risk of COPD. Several studies in animals and humans have provided evidence that MMP-9 (92-kD gelatinase or gelatinase B) and MMP-12 (macrophage metalloelastase) are important in airway inflammation and development of emphysema (Joos et al., 2002a). In addition, MMP-3 (stromelysin-1) can activate several other MMPs and may contribute to airway remodeling (Elshaw et al., 2004).

In view of the important role of MMPs in COPD, it has been hypothesized that genetic variation affecting the expression of MMPs influences the development of COPD. Therefore, the aim of this study was to ascertain if functional polymorphisms in the promoter of MMP-3, MMP-9, and MMP-12 genes are associated with the risk of developing COPD in a Brazilian population of European ancestry.

MATERIAL AND METHODS

Study population

A total of 212 subjects were included in this study. The subjects with COPD (N = 111) were recruited from a Pulmonary Rehabilitation Center (Centro Universitário Feevale, Novo Hamburgo, Brazil; N = 36) and from a general hospital (Complexo Hospitalar Santa Casa de Porto Alegre, Porto Alegre, Brazil; N = 75) as previously described (Gaspar et al., 2004). All subjects with disease had a diagnosis confirmed by spirometry, with FEV_1 <80% and an FEV_1/FVC ratio <0.7 (Pauwels et al., 2001). Pack-years of smoking were calculated at baseline examination as the number of cigarettes per day multiplied by number of years of smoking divided by 20.

The control group consisted of a sample of the blood bank donors, apparently healthy, although no detailed data about their health conditions were obtained. The control group was representative of the Porto Alegre population (1.5 million inhabitants) in terms

of sex and age distribution, and although no data about smoking habits were obtained for this group, the frequency of smokers in this group would be expected to be lower than that of the patients group. Exclusion criteria for both groups were non-Caucasian ethnicity and age less than 40 years old.

All patients answered a standard questionnaire concerning their personal history and smoking history. The study was approved by the Institutional Ethics Committee of the Centro Universitário Feevale. All subjects gave written informed consent for drawing a blood sample for DNA extraction to be used in studies approved by the Hospital Ethics Committee.

DNA analysis

High-molecular weight DNA was extracted from whole blood using a nonenzymatic technique for DNA analysis (Lahiri and Nurnberger Jr., 1991) or from buccal brush using size-fractionated silica particles as the nucleic acid carrier (Boom et al., 1990). The -1171 5A/6A MMP-3 (NCBI Ref. SNP ID: rs3025058), -1562 C/T MMP-9 (rs3918242), and -82 A/G MMP-12 (rs2276109) polymorphisms were analyzed by polymerase chain reaction followed by restriction digestion as previously described (Dalepiane et al., 2007).

Statistical analysis

Allele frequencies were determined by direct counting of the alleles. Departures from Hardy-Weinberg equilibrium and differences between groups were evaluated by the chi-square test. The mean age was compared between groups using the Student *t*-test. Haplotype frequencies were determined using the SNPAnalyzer computer software, which employs an expectation-maximization algorithm (Yoo et al., 2005). A significance level of $P < 0.05$ was considered to be significant.

RESULTS

Brazilian subjects of Caucasian origin (N = 212) were included in this study. The main characteristics of the patients and control subjects are shown in Table 1. There was no difference between COPD patients and control subjects regarding gender ratio, but in relation to age the patients were significantly older than the control group ($P < 0.001$). COPD patients smoked 40 ± 12.5 years (range 4-75 years).

Table 1. Main characteristics of the subjects sampled in this study.

Characteristic	COPD (N = 111)	Controls (N = 101)
Age in years, mean ± SD	64.8 ± 10.1	46.4 ± 8.8
Male gender, N (%)	72 (64.9)	75 (74.3)
Smoking history, N (%; range)		
≤40 pack-years	54 (48.6; 4-40)	-
>40 pack-years	57 (51.4; 41-180)	-
Years smoked, mean ± SD (range)	40 ± 12.5 (4-75)	-
Pack-years, mean ± SD (range)	62 ± 41.5 (4-180)	-

COPD = chronic obstructive pulmonary disease.

The allele and genotype frequencies of the MMP polymorphisms in COPD patients and controls are presented in Table 2. The observed genotype frequencies were in Hardy-Weinberg equilibrium in the total sample and in all subgroups. No significant differences were observed in genotype frequencies between COPD patients and control individuals for any of the polymorphisms analyzed. When we compared the allele frequencies between COPD patients stratified by smoking history (≤40 or >40 pack-years), no statistically significant differences were observed for the polymorphisms analyzed.

Table 2. Allele and genotype frequencies of matrix metalloproteinase (MMP) gene polymorphisms in chronic obstructive pulmonary disease patients and control individuals.

Allele and genotype	Frequency (% in parentheses)		P
	Patients	Controls	
MMP-3 -1171 5A/6A	N = 91	N = 99	
Allele			
5A	89 (48.9)	88 (44.4)	0.38
6A	93 (51.1)	110 (55.6)	
Genotype			
5A/5A	26 (28.6)	23 (23.3)	0.69
5A/6A	37 (40.6)	42 (42.4)	
6A/6A	28 (30.8)	34 (34.3)	
MMP-9 -1562 C/T	N = 89	N = 97	
Allele			
C	162 (91.0)	178 (91.8)	0.80
T	16 (9.0)	16 (8.2)	
Genotype			
CC	74 (83.1)	81 (83.5)	0.91
CT	14 (15.8)	16 (16.5)	
TT	1 (1.1)	-	
MMP-12 -82 A/G	N = 111	N = 101	
Allele			
A	194 (87.4)	186 (92.1)	0.11
G	28 (12.6)	16 (7.9)	
Genotype			
AA	84 (75.7)	85 (84.1)	0.16
AG	26 (23.4)	16 (15.9)	
GG	1 (0.9)	-	

Since the MMP-3 and MMP-12 genes are in the same chromosome cluster (11q22.3), haplotype analysis was undertaken and the estimated haplotype frequencies are presented in Table 3. No significant differences were observed between COPD patients and controls in regard to haplotype frequencies. The haplotype 6A/A (MMP-3 and MMP-12 genes, respectively), which carries the risk alleles, showed similar frequencies between patients and controls.

Table 3. Haplotype frequencies of the matrix metalloproteinase (MMP) gene polymorphism (MMP-3 and MMP-12) in chronic obstructive pulmonary disease patients and control individuals.

Haplotype		Patients	Control individuals
MMP-3	MMP-12	(N = 92)	(N = 96)
5A	A	80 (43.5%)	87 (45.3%)
6A	A	83 (45.1%)	90 (46.9%)
5A	G	10 (5.4%)	-
6A	G	11 (6.0%)	15 (7.8%)

Data are reported as number with percent in parentheses.

DISCUSSION

MMP synthesis and functions are regulated by three major mechanisms including transcriptional activation, post-transcriptional processing, and control of activity by a family of endogenous inhibitors collectively known as tissue inhibitors of metalloproteinases (TIMPs). Transcriptional activation is the primary process regulating MMP activity. There are a number of functional polymorphisms in the MMP gene promoters (Ye, 2000). In this study, we investigated whether polymorphisms in the promoters of MMP-3, -9, and -12 genes were associated with the development of COPD in a Caucasian Brazilian sample, and our results did not find any promoter polymorphism of MMP genes to be a risk factor for developing COPD in white Brazilian smokers.

Functional studies indicate that the -1562 C/T polymorphism has an effect on MMP-9 promoter activity, and that this effect is translated into differences in MMP-9 protein level and activity between individuals of different MMP-9 genotypes (Ye, 2006). MMP-9 is involved in the digestion of extracellular matrix components such as gelatin, collagens and elastin (Atkinson and Senior, 2003). These macromolecules are important in determining the mechanical properties of the lung (Suki et al., 2005). There are some studies, with controversial results, relating polymorphisms in the MMP-9 gene and COPD. An association between polymorphism C-1562T and the risk of developing COPD in Japanese populations was observed (Minematsu et al., 2001; Ito et al., 2005), as well as in a Chinese population (Zhou et al., 2004). On the other hand, this polymorphism was not associated with COPD in a Caucasian population (Joos et al., 2002b).

MMP-3, also called stromelysin-1, shows proteolytic activity on a number of extracellular matrix proteins. In addition, it can activate several other MMPs. This enzyme is believed to play important roles in matrix remodeling. The -1171 5A/6A polymorphism in the promoter has been shown to have an effect on MMP-3 expression and was associated with a number of cardiovascular conditions. The 5A allele has greater activity in driving gene expression than does the 6A allele (Ye, 2006). There are no studies analyzing the MMP-3 gene and the risk of developing COPD. In the present study, we found no association between MMP-3 polymorphism and COPD in Caucasian Brazilian patients. This candidate gene was selected based on the known mechanism in the pathophysiology of this disease.

The -82 A/G polymorphism in the MMP-12 promoter has been shown to have an effect on MMP-12 expression. Carriers of the A allele have higher MMP-12 transcriptional activity than carriers of the G allele (Jormsjö et al., 2000). Studies in animals have provided evidence that the presence of MMP-12 is critical in smoke-induced lung injury (Hautamaki et al., 1997). As it has a potent elastolytic activity, MMP-12 is thought to play a determinant role in the development of COPD and emphysema (Gueders et al., 2006). Recently, increased expression of MMP-12 in alveolar macrophages was observed in COPD patients compared with healthy controls (Montaño et al., 2004). Our study is consistent with previous results in another Caucasian population, which did not find an association between the MMP-12 polymorphism and COPD (Joos et al., 2002b).

The case-control study is a widely used approach, though often producing inconsistent results. This may be because of variation in the definition of cases and controls, underpowered studies, racial differences, and population heterogeneity (Hersh et al., 2005; Wood and Stockley, 2006). COPD patients may have chronic bronchitis, emphysema, small airways disease, or a combination of these, with or without systemic manifestations of the disease. This results in great variety within the patient population (Wood and Stockley, 2006). 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. Many of the COPD association studies have shown inconsistent results in white and Asian populations (Hersh et al., 2005).

It has long been recognized that individual susceptibility to environmental factors varies greatly, but the basis for such variation has remained poorly understood. The effect of a single polymorphism may be subtle, and studies have shown that a combination of several polymorphisms may be more predictive of an individual's response to an exposure. In the present study, no significant differences were observed between COPD patients and controls in regard to MMP-3 and MMP-12 haplotypes. Our results do not discard the possibility that a combination of other polymorphisms with MMP genetic variants may be related to the risk of COPD. Along this line, a polymorphism of the TIMP-2 gene has been documented that may lead to down-regulation of TIMP-2 activity, thereby increasing the activity of MMPs and resulting in the degradation of tissue matrix (Belvisi and Bottomley, 2003).

It is becoming clear that COPD has a complex etiology that involves gene-environment and gene-gene interactions. In this disease, there are likely to be many genes contributing to the overall phenotype, which may have additive or synergistic effects (Wood and Stockley, 2006), and further studies are needed to help us understand the susceptibility mechanism of this disease.

CONCLUSIONS

In summary, our study found no evidence of promoter polymorphisms in MMP-3, -9, and -12 genes being independent risk factors for chronic obstructive pulmonary disease in the Caucasian Brazilian sample studied. However, further studies analyzing other variations in these genes should be conducted to clarify the association of these genes with individual susceptibility to COPD development.

ACKNOWLEDGMENTS

The authors thank Tania A. Weimer for help in the sample collection. Research supported by the Universidade Luterana do Brasil and Centro Universitário Feevale.

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