Genetic polymorphisms of matrix metalloproteinases and protein levels in chronic obstructive pulmonary disease in a Mexican population

Aim: To evaluate association of single nucleotide polymorphisms (SNPs) in the *MMP1*, *MMP2*, *MMP9* and *MMP12* genes and serum MMP-2 and MMP-9 levels in smoking chronic obstructive pulmonary disease (COPD) patients. **Materials & methods:** Genotyping using real-time PCR in 330 smokers with COPD (COPD), 658 smokers without COPD (SNC) and 150 nonsmokers (NCNS), the analysis of samples used was χ^2 test. Using ELISA, the proteins were evaluated. Multiple comparisons were made by ANOVA. **Results:** rs243864 (OR: 7.44; 95% CI: 3.62–15.26) and rs11646643 (OR: 1.58; 95% CI: 1.07–2.34) of the *MMP-2* gene and rs3918253 (OR: 1.72; 95% CI: 1.08–2.71) of the *MMP-9* gene, were associated with the risk of COPD. Serum MMP-2 level in the COPD group was lower compared with SNC ($p < 0.05$). Serum MMP-9 level was elevated in the COPD group compared with SNC ($p < 0.05$). **Conclusion:** Polymorphisms in *MMP2* and *MMP9* but not in *MMP1* and *MMP12* are associated with the risk of COPD in the Mexican mestizo population.

Keywords: COPD • genetic susceptibility • MMP2 • MMP9 • polymorphisms

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation of the airways and lung parenchyma; it is associated with remodeling and degradation of the extracellular matrix (ECM) and loss of elastin fibers in lung tissue [1–3]. It is caused by prolonged exposure to noxious particles and gases; exacerbations and comorbidities contribute to severity and are a significant cause of morbidity and mortality [4–6]. It has been proposed that an interaction exists between genetic and environmental factors that influence individual susceptibility to COPD [7]. The main environmental risk factor is cigarette smoke, which initiates and maintains the inflammatory response in the lungs and airways [8,9]. With respect to genetic factors the extracellular matrix metalloproteinases (MMPs) have an important role due to these proteases are involved in various physiological and pathological processes [10–13]. In humans, 24 enzymes have been described that can alter components of the ECM [14]. MMPs, mainly MMP-1, MMP-2, MMP-9

and MMP-12, play an essential role in repair and remodeling processes in lung tissue [15–17]. Recent genetic studies have implicated MMP genes and endogenous inhibitors called tissue inhibitors of metalloproteinases (TIMPs) in the pathogenesis of COPD; an imbalance of MMPs and TIMPs contributes to disease development [18,19]. Single nucleotide polymorphisms (SNPs) associated with COPD have been described in the *MMP-1*, *MMP-2*, *MMP-9* and *MMP-12* genes in several populations [20,21]. The existence of polymorphisms in genes determines the different levels of expression between individuals who create different disease phenotypes in a given population [22]. In this study, the genotype frequencies of 12 polymorphisms of the *MMP-1*, *MMP-2*, *MMP-9* and *MMP-12* genes were determined in the Mexican mestizo population with the aim of establishing the association of SNPs with disease. Additionally, the serum levels of the MMP-2 and MMP-9 proteins were determined in smokers with and without COPD, as well as in nonsmokers. The additional aim

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of this study was to evaluate whether BMI and pack-years variables are associated with these protein levels for it became multiple comparisons.

Methods

The study observational, comparative cross-sectional was conducted with patients belonging to the Department of Research on Smoking and COPD, National Institute of Respiratory Diseases Ismael Cosío Villegas (INER) of Mexico City. The total population of participants was 1138, of which 988 were smokers: 330 patients with COPD from the COPD Clinic, 658 smokers without COPD (SNC) from the Smoking Cessation Clinic and 150 healthy nonsmoker subjects without clinical evidence of respiratory diseases. Previously, a part of these study groups were analyzed for other genes [23,24]. All participants were Mexican by descent (Mexican parents and grandparents), over 40 years in age and the smokers had smoked at least ten cigarettes a day for 10 years or more. The diagnosis of COPD was based on spirometry data; following the GOLD diagnostic criteria, the control subjects had a postbronchodilator FEV₁/FVC ratio equal to or greater than 70% while the cases diagnosed with COPD had a FEV₁/FVC ratio of less than 70%. The patients were diagnosed by pulmonology specialists at the INER COPD clinic. COPD susceptibility was evaluated for 12 SNP polymorphisms in four MMP genes in the Mexican mestizo population. The associated genotypes in comparison of the cases and controls were determined in the group of 150 nonsmoker subjects, as were the serum levels of metalloproteinases-2 and metalloproteinases-9 encoded by genes in which the associated SNPs are located.

This protocol was evaluated and accepted by the Science and Research Bioethics Committees of the INER in Mexico. All participants were initially invited to participate, and those who agreed signed a letter of informed consent created for that purpose.

DNA extraction

The blood samples were processed in the HLA laboratory of INER to obtain genomic DNA using a commercial kit (BDtract Genomic DNA Isolation Kit, Maxim Biotech, Inc., CA, USA). The DNA was quantified by UV spectrophotometry using NanoDrop 2000 equipment (Thermo Scientific, DE, USA). Contamination with organic compounds and proteins was determined by establishing the ratio of 260/240 and 260/280 readings, respectively. Samples were considered free of contaminants in both cases when the ratio was between 1.7 and 2.0.

Selection of SNPs

The selection process of the evaluated SNPs included a literature review of previous reports of genetic association of SNPs in MMP genes with COPD in Caucasian and Asian populations, using the NCBI (National Center for Biotechnology Information) database and including scientific articles published between 2007 and 2013. For the four included genes, tag SNP selection was performed with HaploView version 4.2, using the minor allele frequency (MAF) ≥10% and r² ≥0.80 in the Caucasian population as a reference. A total of 12 SNPs were selected, and data including the chromosome location and polymorphism base change are shown in Table 1.

Table 1. Single nucleotide polymorphisms selected for genotyping.					
Gene/ chromosome	SNP	Gene location	Allele		Tagged SNPs
			Change	Ancestral	
MMP-1/11	rs470215	3'UTR	A/G	A	rs5854, rs470747
	rs7125062	Intronic	C/T	T	
	rs2071232	Intronic	C/T	T	
MMP-2/16	rs243839	Intronic	A/G	G	rs865094, rs183112, rs243849, rs243843, rs243840, rs243836, rs243834 rs243865, rs243866 rs1477017, rs9302671, rs17301608
	rs243835	Intronic	C/T	C	
	rs243864	(-790) promoter	G/T	T	
	rs11646643	Intronic	A/G	G	
MMP-9/20	rs3918253	Intronic	C/T	C	rs3918256
	rs3918278	Intronic	A/G	G	
MMP-12/11	rs12808148	Intronic	C/T	T	
	rs17368659	Intronic	G/T	G	
	rs2276109	Intronic	A/G	A	

Genotyping

For genotyping by real-time PCR, 3 µl of DNA was used at a concentration of 15 ng/µl. The amplification conditions were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and a final cycle of 60°C for 1 min. The alleles and genotypes of the polymorphisms were characterized by real-time PCR (7300 Real Time PCR System, Applied Biosystems, CA, USA) via allelic discrimination using commercial Taqman probes at 20× concentration (Applied Biosystems). In addition three controls without template (contamination controls) were included for each genotyping plate and 1% of the samples included in the study were genotyped in duplicate as control allele assignment.

Obtaining serum levels of MMP-2 & MMP-9 with ELISA

Based on the results from the genetic association analysis, the serum levels were measured for total MMP-2 protein with commercial ELISA kits (R&D Systems, MN, USA) and for MMP-9 (BioLegend Systems, CA, USA).

Readings were obtained using the iMark™ Microplate Absorbance Reader (Bio-Rad, CA, USA).

Statistical analysis

To determine the SNPs associated with the risk of COPD, the frequencies of the genotypes of the two study groups were compared, and the odds ratio (OR) was calculated with a 95% CI using Epi Info version 7.1.2 (CDC, GA, USA). The ancestral allele was used as a reference for each of the polymorphisms, and population data for the frequencies of the SNPs studied in the Mexican population residing in Los Angeles, California, USA (HapMap-MEX), obtained from the HapMap project (International HapMap Project), were included. Differences between the groups were analyzed via χ^2 test. Linkage disequilibrium ($r^2 > 80$) and analysis of haplotypes were performed with HaploView version 4.2. To determine the relationship of the genotypes with the variables BMI, age, gender, packyears and the ratio in the percentage of the FEV1/FVC ratio post bronchodilator, a binary logistic regression was performed with SPSS 21 (IL, USA). To determine the association of the protein levels with disease, genotype and other variables, factorial ANOVA was used, and comparisons were made between the groups using the Tukey–Kramer test. For the analysis, NCSS version 9 was used (UT, USA).

Results

Characteristics of the study population

The demographic, smoking and lung function characteristics of the study groups are shown in Table 2. There

was a greater ratio of men to women in the COPD group (3.07:1, M:W), whereas in the SNC group, the ratio of women was greater with respect to men (1.06:1, W:M); a similar ratio was observed in the NCNS group (1.83:1, W:M). COPD individuals were older than the SNC individuals ($p < 0.0001$); the opposite pattern was observed with BMI, which was greater in the SNC group compared with that in the COPD group ($p < 0.0001$). The packyears was significantly greater in the COPD group compared with the SNC group ($p < 0.0001$). The mean age of the NCNS individuals was similar to those of the COPD and SNC groups; the BMI of the NCNS group was similar to that of the SNC group. The lung function of the COPD groups was diminished in comparison to that of the SNC group; as expected, the lung function of the NCNS group was normal and differed slightly from that of the SNC group. Variables for which statistically significant differences were found between the groups were considered for logistic regression correction.

Polymorphisms associated with genetic susceptibility to COPD

To determine the genetic susceptibility to COPD, the genotype frequencies of 12 SNPs selected in four MMP genes were compared. Polymorphisms associated with the risk of COPD were found in the *MMP-2* and *MMP-9* genes. No statistically significant associations of risk were found in SNPs evaluated in the *MMP-1* and *MMP-12* genes. All polymorphisms complied with Hardy-Weinberg equilibrium (HW, $p > 0.05$). Three SNPs associated with increased risk of COPD were identified: the homozygous GG genotype rs243864 (10.3 vs 1.51%, OR: 7.44) and the GG genotype rs11646643 (15.45 vs 10.33%, OR: 1.58) of the *MMP-2* gene as well as the TT genotype rs3918253 (11.21 vs 6.83%, OR: 1.72) of *MMP-9*. Interestingly, two SNPs were associated with reduced risk of disease: the heterozygous AG genotype of the rs11646643 polymorphism of the *MMP-2* gene (44.24 vs 54.4%, OR: 0.66) and the CC genotype of the *MMP-9* gene (49.09 vs 57.75%); however, only two genotypes retain association after Bonferroni's correction (rs243864/GG $p = 1.20E-09$ and rs11646643/AG $p = 3.08E-02$).

The frequencies of all of the genotypes evaluated as well as those described for HapMap Mexican mestizos are found in Table 3. Supplementary Table 2 includes p-values, OR and 95% CI for significant and not significant data. Based on the previously described results in the genetic association analysis, genotyping was conducted on the two SNPs most strongly associated with COPD risk (*MMP-2* rs243864 and *MMP-9* rs3918253) in a population comparison group composed of 150 nonsmokers without COPD and

Table 2. Demographic data of the study subjects.

Characteristics	COPD (n = 330)	SNC (n = 658)	NCNS (n = 150)	p-value
Gender (%), M/F	76/24	48/52	35/64	<0.0001
Age (years)	67 ± 9	53 ± 9	62 ± 13	<0.0001
BMI (kg/m ²)	25.47 ± 5	27.12 ± 4	28 ± 6	<0.0001
Packyears	40 ± 38	27 ± 22	-	<0.0001
Lung function (%):				
– FEV ₁ (predicted)	60 ± 27	98 ± 49	90 ± 19	<0.0001
– FVC (predicted)	83 ± 25	96 ± 17	84 ± 18	<0.0001
– FEV ₁ /FVC	56 ± 15	79 ± 8	89 ± 11	<0.0001

Data are reported as the media ± SD. The p-value for comparing COPD and SNC were by statistical test χ^2 test. Smokers with COPD (COPD), smokers without COPD (SNC) and nonsmokers (NCNS). BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; FEV₁: Forced expiratory volume in 1 s; FVC: Forced vital capacity; M/F: Male/female.

without exposure to smoke from burning biomass. The genotypic frequency (GF) percentages for the rs243864 polymorphism of the *MMP-2* gene were TT (56.66%), GT (36.66%) and GG (7.03%) and were CC (59.33%), CT (24%) and TT (16.66%) for the rs3918253 polymorphism of the *MMP-9* gene.

Comparison of frequencies in the study groups with population values

The genotype frequencies of the 12 SNPs in four MMP genes of the SNC group were compared with the described HapMap frequencies in Mexicans born in Los Angeles. It was identified that the frequency of the CT genotype of the rs7125062 polymorphism of the *MMP-1* gene in the SNC group was slightly reduced when compared with the population data from HapMap (49.24 vs 58.0%), contrary to what was observed with the frequency of the TT genotype of the same polymorphism, which was slightly elevated in the SNC group. For the *MMP-2* gene, the CC genotype frequency of the rs243835 polymorphism was different from the reported HapMap frequency (26.59 vs 38%), as was the CT genotype frequency of the rs3918253 polymorphism in the *MMP-9* gene (35.41 vs 25%). Interestingly, the CC genotype frequency of the rs12808148 polymorphism in the *MMP-12* gene of the SNC group that represents Mexican mestizos by descent was 12%, but that genotype was not reported in the resident Mexican population in Los Angeles, California, by the HapMap project.

A binary logistic regression was performed considering each of the polymorphisms and their respective genotypes as dependent variables and the post bronchodilator lung function value as an independent variable. An association was only found for rs3918253 of the *MMP-9* gene under the CC versus CT + TT model, where a significant p value ($p = 0.001$, $\beta = -0.017$, 95% CI: 0.97–0.99) was obtained with the post bronchodilator FEV₁/FVC ratio.

SNP haplotypes in MMP genes

Haplotype analysis was performed on polymorphisms in the *MMP-1*, *MMP-2*, *MMP-9* and *MMP-12* genes; they were analyzed by chromosomal location. No haplotypes were found to be associated with EPOC ($r^2 < 80$); graphs of the haplotypes formed for *MMP-1*, *MMP-2*, *MMP-9* and *MMP-12* genes are shown in Supplementary Figure 1.

Relationship of the serum levels of the MMP-2 & MMP-9 proteins with COPD

The serum levels of the MMP-2 and MMP-9 proteins of the COPD, SNC and NCNS groups were measured by ELISA using commercial kits. The serum level of MMP-2 of the COPD group was significantly lower compared with that of the SNC group (446.72 vs 983.44 ng/ml, $p < 0.05$). There was a statistically significant difference between the MMP-2 serum levels of the SNC and NCNS groups (983.44 vs 660.33 ng/ml, $p < 0.05$), as shown in Figure 1; in contrast, the serum level of the MMP-9 protein was elevated in the COPD group compared with that of the SNC group (1468.69 vs 771.64 ng/ml, $p < 0.05$). There was a statistically significant difference between the SNC and NCNS groups (771.64 ng/ml vs 1,394.39 ng/ml, $p < 0.05$). No association was found between the serum levels of MMP-2 and MMP-9 proteins and other variables.

Discussion

In this genetic association study, we showed that three (rs243864, rs11646643 and rs3918253) of the 12 evaluated polymorphisms in the *MMP-1*, *MMP-2*, *MMP-9* and *MMP-12* genes are associated with genetic susceptibility for COPD in the Mexican mestizo population. GG rs243864 (OR: 7.44) and GG rs11646643 (OR: 1.58) in *MMP-2* and TT rs3918253 (OR: 1.72) in the *MMP-9* gene are associated with the risk of COPD.

Table 3. Genotype frequencies of *MMP-1*, *MMP-2*, *MMP-9*, *MMP-12* polymorphisms among chronic obstructive pulmonary disease and smokers nonchronic obstructive pulmonary disease groups.

Gen/SNP	COPD (n = 330)		SNC (n = 658)		p-value	OR	CI 95%	HAPMAP-MEX
	n	GF (%)	n	GF (%)				GF (%) n = 100
MMP-1								
rs470215								
AA	200	60.6	400	60.79				59.7
AG	113	34.24	238	36.17				39.0
GG	17	5.15	20	3.03				1.3
rs7125062								
CT	173	52.42	324	49.24				58.0
TT	96	29.09	205	31.15				24.0
CC	61	18.48	129	19.6				18.0
rs2071232								
TT	189	57.27	332	50.45				54.0
CT	117	35.45	278	42.24				40.0
CC	24	7.27	48	7.29				6.0
MMP-2								
rs243839								
AA	153	46.36	329	50				54.0
AG	139	42.12	264	40.12				38.0
GG	38	11.51	65	9.87				8.0
rs243835								
CT	177	53.63	330	50.15				44.0
CC	87	26.36	175	26.59				38.0
TT	66	20	153	23.25				18.0
rs243864								
TT	199	60.3	426	64.74				58.0
GT	97	29.39	222	33.73				40.0
GG	34	10.3	10	1.51	1.00 × 10 ⁻¹⁰	7.44	3.62–15.26	2.0
rs11646643								
AG	146	44.24	358	54.4	3.20 × 10 ⁻⁰³	0.66	0.50–0.86	62.0
AA	133	40.3	232	35.25				30.0
GG	51	15.45	68	10.33	2.58 × 10 ⁻⁰²	1.58	1.07–2.34	8.0
MMP-9								
rs3918253								
CC	162	49.09	380	57.75	1.20 × 10 ⁻⁰²	0.6	0.46–0.79	64.5
CT	131	39.69	233	35.41				25.0
TT	37	11.21	45	6.83	2.58 × 10 ⁻⁰²	1.72	1.08–2.71	10.5
rs3918278								
GG	266	80.6	598	91.03				89.6
AG	62	18.78	59	8.96				9.5
AA	2	0.6	1	0.15				0.9

Table 3. Genotype frequencies of *MMP-1*, *MMP-2*, *MMP-9*, *MMP-12* polymorphisms among chronic obstructive pulmonary disease and smokers nonchronic obstructive pulmonary disease groups (cont.).

Gen/SNP	COPD (n = 330)		SNC (n = 658)		p-value	OR	CI 95%	HAPMAP-MEX
	n	GF (%)	n	GF (%)				GF (%) n = 100
MMP-12								
rs12808148								
TT	262	79.39	555	84.34				84.0
CT	66	20	95	14.43				16.0
CC	2	0.6	8	0.12				–
rs17368659								
GG	280	84.84	586	89.05				84.0
GT	49	14.84	69	10.48				14.0
TT	1	0.3	3	0.45				2.0
rs2276109								
AA	287	86.96	599	91.03				84.0
AG	42	12.72	57	8.66				14.0
GG	1	0.3	2	0.3				2.0

The prevalence of disease is greater in men than in women; in this study, we found a similar pattern (3.07:1, M:W) to that described in the PLATINO study [25].

In the pathogenesis of COPD, there is an increase in neutrophils and macrophages, innate immunity cells that express proteolytic enzymes such as extracellular matrix metalloproteinases (MMPs), mainly MMP-1, MMP-2, MMP-9 and MMP-12. MMPs contribute to chronic inflammation, the development of COPD and the presence of emphysema [26]. In patients with emphysema, there is increased production of MMP-2 and MMP-9 (gelatinases A and B, respectively) in the lung parenchyma, resulting in degradation and remodeling of this tissue associated with chronic inflammation [27-29]. Different studies have evaluated the involvement of genetic factors in the susceptibility and severity of COPD [30-33]; the most clearly associated factor, genetic deficiency of alpha-1 antitrypsin, is highly prevalent among individuals of northern Europe [34]. However, in Mexican mestizos, the frequency of polymorphisms associated with this deficiency is low [35], suggesting the existence of other genetic factors associated with risk.

To date, there are no genetic association studies of rs243864 with COPD; to our knowledge we are the first to demonstrate the strong genetic association of rs11646643 and rs243864 polymorphisms (-790 T/G) with the risk of developing COPD in the Mexican mestizo population. The latter polymorphism is located in the GATA-1 site (CTATCT) of the promoter region of the *MMP-2* gene [36], which has binding sites for the transcription factors AP-2, p53, Sp1 and Sp3 [37]. These transcription factors regulate the transcription

rate of the gene and, therefore, the expression of the protease. Additionally, rs243864 is a tag SNP of the rs243865 (-1306 C/T) and rs243866 (-1575G/A) polymorphisms [38], which are also found in the gene promoter. Interestingly, we found that the GG genotype of rs243864 has the highest OR value (7.44) among associated SNPs. Another finding was that the TT genotype of the rs3918253 polymorphism of the *MMP-9* gene (OR: 1.72) is also associated with the risk of COPD; this genotype is an intronic variant and a tag SNP of the rs3918256 polymorphism, which is reported to not have a genetic association with COPD in the Caucasian population [20]. There are no previous studies of rs3918253 genetic association with COPD; our results are the first to indicate an association of susceptibility of the *MMP-9* gene to disease. However, other SNPs in the promoter of the *MMP-9* gene have been reported, among which is rs3918242 (-1562 C/T), which has been associated with emphysema in the Japanese population [41]. Furthermore, a meta-analysis conducted by Chen *et al.* found that this polymorphism increases the risk of COPD in the Asian population but not in Caucasians [42]. Another study in the Caucasian population found no genetic association of the rs3918242 polymorphism with loss of lung function [43]. These findings indicate that SNPs have a different impact on different populations. In addition, genetic factors interact with environmental factors to influence individual susceptibility. A similar phenomenon could be taking place with the genotype frequencies of the group of smokers without disease (SNC) compared with those described for HapMap Mexican mestizos. The CT genotype of

Figure 1. Total MMP-2 and MMP-9 protein levels measured by ELISA in smokers with chronic obstructive pulmonary disease (COPD), smokers without COPD (SNC) and nonsmokers (NCNS). Analysis of variance (ANOVA; Tukey–Kramer’s) comparing protein levels between the groups. The results are expressed as the mean $\pm$ SD.

* $p < 0.05$ significant difference between COPD and SNC for MMP-2 and MMP-9.

** $p < 0.05$ denotes a significant difference between SNC and NCNS for MMP-2 and MMP-9.

rs3918253 in the *MMP-9* gene shows a 10% difference between the two populations (35.41 vs 25%). However, it should be taken into consideration that for the population and variant consulted in the HapMap project, only 100 Mexican residents were evaluated in Los Angeles (CA, USA), that is, individuals with Mexican ancestry; our study included a total of 658 smokers without COPD. The AG genotype of rs243864 and the CC genotype of rs3918253 of the *MMP-2* and *MMP-9* genes, respectively, are associated with decreased risk of disease (OR: 0.6); both represent the highest frequency of genotypes, suggesting they could positively influence smokers carrying these alleles. In our study, we found a reduced FEV₁/FVC (%) post bronchodilator ratio in homozygous TT carriers for rs3918253 of the *MMP-9* gene, the same genotype that is associated with risk of COPD (OR: 1.72). These findings indicate some involvement of this polymorphism in the pathogenesis of COPD in our population.

It was recently reported that there is systemic inflammation in patients with COPD [42], which is mediated primarily by an increase in systemic circulation of pro-inflammatory cytokines [44,45]; one report suggested that MMPs in serum are potential repair biomarkers

in emphysema [46]. In our study, the serum levels of the MMP-2 and MMP-9 proteins, both of which are gelatinases from the MMP group, were evaluated in the COPD, SNC and NCNS groups; the MMP-9 protein was related through its function mainly in the remodeling of basement membranes, especially of collagen types I and IV and elastic fibers [47]. In patients with COPD, it was shown that the MMP-9 protein increases in serum and correlates with the serum levels of its major endogenous inhibitor, TIMP-1 [48]. This finding is similar to what occurred in our study, in which we found increased serum levels of MMP-9 protein in the COPD group compared with the serum levels observed in the SNC group. Notably, we did not find any relationship of these levels with genotypes or other variables.

Regarding MMP-2, it is involved in remodeling of the extracellular matrix in airways and lung interstitium by degrading molecules such as collagen type I, III, IV, V, VII and X, gelatin, fibronectin, laminin and aggrecan [49]. In our study, the serum levels of MMP-2 were found to decrease in the COPD group compared with those in the SNC group. Similar to our finding, in 2010, Gosselink *et al.* found that mRNA expression of the *MMP-2* gene and activity of gelatinase

decreases in lung tissue and small airways, related to a decline in FEV1. A second study showed that MMP-2 decreases in bronchial epithelial cells of patients with severe COPD [50], contrary to the reports of Segura-Valdez *et al.* in 2013, who found an increase in the number of alveolar macrophages and epithelial cells labeled with MMP-2 by immunohistochemistry in patients with COPD. MMP-2 has the ability to cleave monocyte chemoattractant protein-3 (MCP-3), allowing it to bind to chemotactic receptors 1, 2 and 3 without activating them. This feature would suggest that the decrease in MMP-2 contributes to a persistent inflammatory response in COPD by not allowing modification of MCP-3 [50]. Finally, we should consider that cigarette smoke induces expression of other proteinases and MMPs, which despite the decrease in MMP-2 in the COPD group, could ultimately be causing the characteristic damage of the disease [18,26] in a manner independent from MMP-2.

A limitation of our study is that the population of Mexican mestizos by descent has primarily Amerindian and Caucasian genetic contributions in different proportions, which could cause the associated SNP findings to not necessarily be replicated in other populations. We found five SNPs associated with COPD; however, only two SNPs retain association after Bonferroni's correction (rs243864/GG and rs11646643/AG). It is desirable that SNPs associated with COPD in the *MMP-2* and *MMP-9* genes be evaluated in one or more populations by independent groups, particularly in other mestizo populations and thus strengthen the idea that the participation of these SNPs in the risk of COPD is real.

Our results contribute to the knowledge of genetic factors in candidate genes in the development of COPD. Ultimately, trials are required to evaluate these polymorphisms as potential markers of clinical phenotypes of disease, considering that COPD is an inflammatory disease that not only affects the airways but also shows systemic inflammation associated with increased morbidity and mortality.

Future perspective

Currently, the genetic association studies provide information on the genotype frequencies of SNPs in candidate genes for multifactorial diseases, such as COPD, and seek to know the expression of the gene or protein. Our

study measured serum MMP proteins associated with COPD; although unrelated to the genotype or other variables, was found serum levels were modified in the study groups; in the future, next studies should include identification of associated SNPs, gene expression assays, protein allocation and biological function.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/BMM.15.75

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This protocol was evaluated and accepted by the Science and Research Bioethics Committees of the INER in Mexico. All participants were initially invited to participate, and those who agreed signed a letter of informed consent created for that purpose.

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Executive summary

- Polymorphisms of the *MMP-2* and *MMP-9* genes are associated with risk of chronic obstructive pulmonary disease in the Mexican mestizo population.
- There are differences in the serum levels of MMP2 and MMP9 proteins between the study groups.
- Our study provides the knowledge that polymorphisms and the proteins associated genes influence the presence of chronic obstructive pulmonary disease.

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