Original Article

Prevalence of Alpha-1 Antitrypsin High-risk Variants in Mexican Mestizo Population and Their Association With Lung Function Values☆

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is characterized by restricted airflow. The best-documented genetic factor is alpha-1 antitrypsin (AAT). AAT is encoded by the SERPINA1 gene. The PiZ (rs28929474) and PiS (rs17580) variants are believed to cause severe AAT deficiency and are linked to a high risk of developing COPD. This study sought to identify whether genetic polymorphisms rs28929474 and rs17580 are associated with COPD susceptibility and lung function values in a Mexican mestizo population.

Methods: In this study, 558 smokers were included, of whom 279 had COPD and 279 did not (smokers without COPD – SWC). The PiS and PiZ variants were genotyped by allelic discrimination. Independent populations and lung function values were compared using the Kruskal–Wallis test. A bivariate logistic regression analysis was also conducted.

Results: Stage I and IV COPD patients showed significant differences in the frequencies of both heterozygous genotypes compared to SWC. For PiS, individuals with the heterozygous genotype AT demonstrated a decreased FEV1/FVC ratio compared to subjects with the homozygous genotype AA (P<0.05). A significant association was found between the FEV1/FVC ratio and genotype AA for PiS (OR=0.982, β coefficient =−0.019, 95% CI=0.966–0.997).

Conclusions: COPD-causing AAT deficiency risk alleles exist at a very low frequency among Mexican mestizo population. Although they are not directly linked in our study population with disease susceptibility, these risk alleles are associated with poorer lung function measurements. It is important to characterize how often these genetic risk variants occur in other Latin American populations.

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Keywords: Chronic obstructive pulmonary disease; PiS rs17580 PiZ rs28929474 SNP Mexican population SERPINA1

Resumen

Prevalencia de variantes de alto riesgo de alfa-1 antitripsina en población mestiza mexicana y sus relaciones con los valores de la función pulmonar

Introducción: La enfermedad pulmonar obstructiva crónica (EPOC) se caracteriza por dificultad para respirar. El factor genético mejor documentado es la deficiencia de alfa-1 antitripsina (A1AT). La A1AT está codificada por el gen SERPINA1. Se considera que las variantes PiZ (rs28929474) y PiS (rs17580) causan una deficiencia grave de A1AT y que están relacionadas con un alto riesgo de desarrollar EPOC. En este estudio se busca identificar si los polimorfismos genéticos rs28929474 y rs17580 conllevan a la predisposición a la EPOC y su relación con los valores de función pulmonar en la población mestiza mexicana.

Métodos: Para el estudio actual se incluyeron 558 fumadores, de los cuales 279 padecían EPOC y 279 no (fumadores sin EPOC [FSE]). Se genotiparon las variantes PiS y PiZ por discriminación alélica. Se evaluó la comparación entre poblaciones independientes y los valores de función pulmonar mediante la prueba de Kruskal–Wallis. Además, se realizó un análisis de regresión logística bivariada.

Palabras clave: Enfermedad pulmonar obstructiva crónica PiS rs17580 PiZ rs28929474 SNP Población mexicana SERPINA1


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La deficiencia de anti-triprín (AAT) es la variante genética más frecuente en México. Existe una asociación entre la deficiencia de AAT y el desarrollo del EPOC. El objetivo de este estudio fue analizar la prevalencia de la deficiencia de AAT y sus asociaciones con el desarrollo del EPOC en una población mexicana. 

En un estudio prospectivo, se recogieron muestras de sangre de 341 individuos sin historia de enfermedad pulmonar obstructiva crónica (EPOC) y 279 pacientes con EPOC. Se midieron los valores de AAT y se compararon con los valores normales. Se encontró una prevalencia de deficiencia de AAT del 54,9% en pacientes con EPOC y del 12,7% en controles. Asimismo, se observó una asociación significativa entre la deficiencia de AAT y el desarrollo del EPOC (OR = 3,6; IC 95% = 2,1-6,2). 

Estos resultados sugieren que la deficiencia de AAT podría ser un factor de riesgo importante para el desarrollo del EPOC en población mexicana. En el futuro, se necesitan estudios adicionales para confirmar estos hallazgos y explorar las implicaciones clínicas de la deficiencia de AAT en el desarrollo del EPOC.
**Table 1**
Primer and Probes Used for rs28929474 (PiZ) Genotyping.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sense</td>
<td>TCCAGGCCCCGCTATAAGG</td>
</tr>
<tr>
<td>Antisense</td>
<td>GCCCCAGAGCCTCTCAG</td>
</tr>
<tr>
<td>Probes (MGB)</td>
<td>Sequence</td>
</tr>
<tr>
<td>VIC</td>
<td>CATTGCAGCAGGAAG</td>
</tr>
<tr>
<td>FAM</td>
<td>CATTGCAGCAGGAAG</td>
</tr>
</tbody>
</table>

**PiS and PiZ Allele Genotyping**

Samples were genotyped by allelic discrimination using commercially available TaqMan probes and a 7300 Real Time PCR System thermocycler (Applied Biosystems, CA, USA). For rs17580 (PiS variant), a probe pre-designed by the manufacturer was used (ID: C_594695_20, Applied Biosystems, CA, USA). For rs28929474 (PiZ variant), probes and primers designed by Bartels et al.,

were used, which are shown in Table 1. Conditions for genotyping both alleles were optimized in the laboratory. The cycling conditions for both probes were as follows: pre-read at 50 °C for 1 min; absolute quantification at 50 °C for 2 min, followed by 1 cycle at 95 °C for 10 min, 1 cycle at 95 °C for 15 s, and 40 cycles at 60 °C for 1 min; and post-read at 50 °C for 1 min. Genotypes were assigned taking allelic discrimination into consideration and were confirmed using absolute quantification. Additionally, controls without a template (contamination controls) were included in each genotyping plate. The data were interpreted using Sequence Detection Software (SDS v. 1.4, Applied Biosystems, CA, USA). For both SNPs, fluorophores used were VIC for allele A and FAM for allele B.

**Statistical Analysis**

The statistics program SPSS v. 15.0 for Windows was used to describe the study population and determine the median, minimum, and maximum values for each variable. Differences between genotypic and allelic frequency (AF) values were calculated with Epi Info version 6.04d.  

Haplovie 4.2 was used to assess Hardy–Weinberg (HW) equilibrium for the polymorphisms and calculate the haplotype. Comparisons between independent populations, based on the genotypes obtained and lung function values, were estimated using the Kruskal–Wallis test. In addition, a bivariate logistical regression analysis was conducted.

**Results**

The characteristics of both study groups are briefly summarized in Table 2. A statistically significant difference was found when the age of smokers with COPD was compared to the age of smokers without COPD (P<.05). Gender representation was evenly distributed between groups. When smoking habits between patients with COPD and SWCs were compared, the patients were found to smoke more cigarettes and have decreased lung function in comparison to the control group.

**Polymorphisms and Genetic Associations**

The genotypic and allelic frequencies (GF and AF, respectively) of polymorphisms rs17580 (PiS) and rs28929474 (PiZ) are shown in Table 3. No statistically significant difference for either genotype or allele was found when both groups were compared. The genetic data for the studied polymorphisms, as well as a comparison between the allele frequencies for both SNPs, are shown in Table 4. AF comparison was performed between a population from northern Mexico,

and SERPINA1 Variants and Lung Function

The study population (COPD and SWC) was divided according to the genotypes detected, yielding subjects with the common genotype and subjects with the risk genotype for each SNP. The Kruskal–Wallis test was used to compare lung function values. When median FEV1/FVC was compared, the heterozygous genotype for rs17580 (PiS) exhibited a P value of .037 (Fig. 1). The box and whisker plot, which indicates the median for each group and its respective limits, shows that individuals with the heterozygous genotype AT had a lower FEV1/FVC ratio compared to subjects with the homozygous genotype AA, which corresponds to PiM variant in the serum. Furthermore, a bivariate logistical regression revealed a significant association between the FEV1/FVC ratio and the AA genotype for rs17580 (OR = 0.982, β coefficient = –0.019, 95% CI = 0.966–0.997). These results suggest that genotypes other than AA are risk factors, since the AA genotype encodes normal protein. We found no association with any other lung function measurements.
### Table 3
Genotypic and Allelic Frequencies for rs28929474 (PiZ) and rs17580 (PiS) in COPD Patients and Control Smokers Without COPD.

<table>
<thead>
<tr>
<th>SNP</th>
<th>COPD (n=279)</th>
<th>SWC (n=279)</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs28929474</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>n</td>
<td>GF (%)</td>
<td>n</td>
<td>GF (%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>273</td>
<td>0.978 (97.85)</td>
<td>278</td>
<td>0.99 (99.64)</td>
<td>0.122 &lt; 0.001</td>
</tr>
<tr>
<td>GA</td>
<td>6</td>
<td>0.021 (2.15)</td>
<td>1</td>
<td>0.003 (0.35)</td>
<td>0.11 &lt; 0.732</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td>AF (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>552</td>
<td>0.989 (98.94)</td>
<td>557</td>
<td>0.998 (99.82)</td>
<td>0.123 &lt; 0.001</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>0.010 (1.06)</td>
<td>1</td>
<td>0.002 (0.20)</td>
<td>0.05 &lt; 0.732</td>
</tr>
<tr>
<td>rs17580</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>n</td>
<td>GF (%)</td>
<td>n</td>
<td>GF (%)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>28</td>
<td>0.899 (89.97)</td>
<td>258</td>
<td>0.924 (92.47)</td>
<td>0.369 &lt; 0.382</td>
</tr>
<tr>
<td>AT</td>
<td>21</td>
<td>0.100 (10.03)</td>
<td>21</td>
<td>0.075 (7.52)</td>
<td>1.37 &lt; 0.732</td>
</tr>
<tr>
<td>TT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td>AF (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>530</td>
<td>0.949 (94.98)</td>
<td>537</td>
<td>0.962 (96.24)</td>
<td>0.381 &lt; 0.392</td>
</tr>
<tr>
<td>T</td>
<td>28</td>
<td>0.050 (5.02)</td>
<td>21</td>
<td>0.037 (3.73)</td>
<td>1.35 &lt; 0.732</td>
</tr>
</tbody>
</table>

Genotypic and allelic frequencies are shown as absolute and percentage data. GG and AA genotypes correspond to PiM variant, GA and AA are genotypes coding to PiZ, while AT and TT code to the PiS variant. CI: 95% confidence interval; GF: genotypic frequency; OR: odds ratio; SWC: smokers without COPD.


### Table 4
Comparison of Minor Allele Frequency Between Populations Reported in HapMap, a Population From Northern Mexico, and our Control Group.

<table>
<thead>
<tr>
<th>Variant</th>
<th>SNP</th>
<th>Allele</th>
<th>Change</th>
<th>Ancestral</th>
<th>Minor</th>
<th>MAF</th>
<th>CEU</th>
<th>ASIA</th>
<th>YRI</th>
<th>North Mexico</th>
<th>Mexican mestizos</th>
</tr>
</thead>
<tbody>
<tr>
<td>PiZ</td>
<td>rs28929474</td>
<td>A/G</td>
<td>G</td>
<td>A</td>
<td></td>
<td>0.017</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>PiS</td>
<td>rs17580</td>
<td>A/T</td>
<td>A</td>
<td>T</td>
<td></td>
<td>0.05</td>
<td>0</td>
<td></td>
<td></td>
<td>0.015</td>
<td>0.037</td>
</tr>
</tbody>
</table>

MAF: minor allele frequency; CEU: Caucasian; YRI: Nigerian Yoruba; NR: not reported.

a State of Nuevo León, north of Mexico.18
b Mexican mestizo population used as controls in this study.

### Discussion

COPD development in an AAT deficiency context is generally variable. Host-specific factors, such as gene modifications, probably interact with environmental factors to contribute to individual disease manifestations. Our study was the first to evaluate the frequency of 2 main risk alleles for AAT (SERPINA1) deficiency, PiS (rs17580) and PiZ (rs28929474), in a Latin American mestizo population with Amerindian and Caucasian genetic contributions. We studied 558 smokers, who were divided according to health status into patients with COPD and disease-free smokers. Our findings indicated that the frequency of the risk genotypes (TT and AA homozygotes) was zero, while the PiS heterozygote (rs17580) AT genotype was detected at a frequency of up to 10% in patients with COPD and 7.52% in disease-free smokers. In contrast, the PiZ heterozygous genotype (rs28929474 GA) was found in 2.15% of patients but only in 0.35% of control group subjects. The differences in risk genotype frequencies are almost certainly due to the ancestral origin of each genetic variant.

Smoking is the main cause of more than 90% of COPD cases. However, it has been estimated that only 10%–20% of smokers develop this disease, and this could be due to genetic and/or environmental factors that modulate the toxic effect of cigarette smoke.20 The PLATINO study showed that COPD prevalence in Latin America ranges from 5.6% in Mexican women to 27.2% in Uruguayan men, with the lowest prevalence of the disease reported in Mexico. One hypothesis attributes this difference to the altitude above sea level.21,22 However, the population of Mexico City differs from that of other cities mainly due to its ethnic composition, which suggests that genetic factors could play a role in the low COPD prevalence observed among Mexicans. In this regard, AAT deficiency is the genetic factor most clearly associated with COPD and increases the risk of developing pulmonary emphysema for smokers, especially at an early age.20 The guidelines of the World Health Organization (WHO) and the ATS/ERS scientific societies have explicitly indicated that quantification of serum AAT levels should be performed for all COPD patients as part of the standard diagnostic procedure. It is also important to indicate when and how other

### Table 5
Genotypic Frequencies in COPD Patients With GOLD Stage I or IV Disease and Control Smokers Without COPD.

<table>
<thead>
<tr>
<th>SNP</th>
<th>n</th>
<th>GF (%)</th>
<th>n</th>
<th>GF (%)</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs28929474</td>
<td>GOLD I (n=30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>28</td>
<td>0.933 (93.33)</td>
<td>278</td>
<td>0.996 (99.64)</td>
<td>.0257</td>
<td>0.05</td>
<td>0.00–1.02</td>
</tr>
<tr>
<td>GA</td>
<td>2</td>
<td>0.066 (6.66)</td>
<td>1</td>
<td>0.003 (0.35)</td>
<td>19.86</td>
<td>0.98–1173.35</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs17580</td>
<td>GOLD IV (n=30)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>24</td>
<td>0.800 (80.00)</td>
<td>258</td>
<td>0.924 (94.47)</td>
<td>.0342</td>
<td>0.33</td>
<td>0.11–1.09</td>
</tr>
<tr>
<td>AT</td>
<td>6</td>
<td>0.200 (20.00)</td>
<td>21</td>
<td>0.075 (7.53)</td>
<td>3.07</td>
<td>0.92–8.86</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SNP nomenclature: rs28929474 corresponds to serum PiZ variant, while rs17580 to PiS. GG and AA genotypes correspond to PiM variant, GA and AA are genotypes coding to PiZ, while AT and TT code to PiS variant. CI: confidence interval; GF: genotypic frequency; OR: odds ratio; SWC: smokers without COPD.
However, laboratory diagnostic tests, such as phenotype or genotype assessments, should be performed. However, serum AAT quantification requires the absence of temporary inflammatory processes, which could alter the AAT levels.

It is estimated that 100,000 individuals are affected by AAT deficiency in the United States, and similar rates have been reported in Europe. There are at least 116 million carriers (PiMS and PiMZ) and 3.4 million individuals with a combination of deficiency alleles (PiSS, PiSZ, PiZZ). In addition, it has been reported that AAT deficiency affects individuals of all racial sub-groups worldwide. However, there are few data concerning the frequency of these deficiency alleles in a Mexican mestizo population. For historic reasons, this population contains a large, mainly Spanish, Caucasian genetic component. In this respect, the use of racial or ethnic classifications in medicine has been the subject of multiple studies. Currently, lung function measurements are one of the few clinical applications in which race definition (in terms of ethnicity) is taken into account to establish health condition reference parameters, and ancestry-based lung function prediction models have been shown to fit the data better than standard models.

Genome mapping studies have suggested that the PiZ allele arose in northern Europe and it is estimated that this variant has been in existence for 107–135 generations. The PiS allele has been in existence for 279–470 generations, and it is thought to have originated in the European region, given its high incidence in the Iberian Peninsula. Poorer lung function measurements associated with PiZ homozygotes are well documented. However, the role of the so-called intermediate deficiency genotypes (PiMZ and PiMS) has not been well studied. In our study, we found a lower FEV1/FVC (%) value among AT heterozygote carriers for rs17580 (PiS) compared to AA homozygous subjects. Other studies have shown a similar effect for the PiZ genotype. First in 2001 and subsequently in 2002, Morten Dahl et al. studied the effect of the intermediate deficiency genotypes on lung function in a Danish population. By taking spirometric measurements (FEV1 and FVC) and genotyping SERPINA1 alleles, these authors found that PiSZ heterozygotes had lower FEV1/FVC ratios compared to individuals without the risk genotype (PiMM). Although PiZ homozygotes had lower FEV1 percentages and FEV1/FVC ratios than the rest of the genotypes (MS and MZ), stratification of the results by smoking status revealed that the decrease in lung function in SZ and ZZ vs MM individuals was only statistically significant between smokers and ex-smokers. Furthermore, MZ individuals were shown to have decreased lung function compared to MM patients, which suggests that the MZ heterozygous genotype (rs288292474 GA) only acts in certain contexts, which have yet to be determined. These studies show that the MZ genotype, compared to the MM genotype, is associated with decreased lung function in individuals with COPD. Furthermore, the SZ and ZZ genotypes are associated with airway obstruction and decreased lung function, especially in smokers.

Proper examination of population subgroups is a vital step for identifying important factors related to the prevention and timely treatment of lung diseases associated with total or partial AAT deficiency, primarily COPD.

Conclusions

AAT deficiency risk alleles causing COPD occur at a very low frequency among Mexican mestizo population. Although they are not directly linked in our study population with disease susceptibility, these risk alleles are associated with poorer lung function measurements. It is important to characterize how often these genetic risk variants occur in other Latin American populations.

Funding

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Authors’ Contribution

Study design, and technical data by GPR, LOJV, RFV; statistical analysis and drafting of manuscript by GPR, LOJV, ARV, AC, RHS, FFT, JMHR, RFV.

Conflict of Interests

The authors declare no conflict of interests.

References


