



## Original Article

## HLA-DRB1 Alleles are Associated With COPD in a Latin American Admixed Population


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## ABSTRACT

**Introduction:** While the molecular mechanisms of COPD pathogenesis remain obscure, there is mounting evidence supporting a key role for autoimmunity. Although human leukocyte antigens (HLA) alleles have been repeatedly associated with autoimmune processes, the relation between HLA and COPD remains largely unexplored, especially in Latin American (LA) populations. Consequently, this study aimed to investigate the presence of HLA class I and II alleles in COPD patients and healthy controls in a LA population with admixed ancestry.

**Methods:** COPD patients ( $n = 214$ ) and age-matched controls ( $n = 193$ ) were genotyped using the Illumina Infinium Global Screening Array. The classic HLA alleles were imputed using HLA Genotype Imputation with Attribute Bagging (HIBAG) and the Hispanic reference panel. Finally, the distribution of HLA-DRB1 alleles was reexamined in 510 randomly recruited unrelated volunteers.

**Results:** COPD patients showed a higher HLA-DRB1\*01:02 allele frequency (6.54%) than healthy controls (3.27%,  $p = 0.04$ , OR = 2.07). HLA-DRB1\*01:02 was also significantly associated with FEV<sub>1</sub> ( $p = 0.04$ ) and oxygen saturation ( $p = 0.02$ ), and the FEV<sub>1</sub>/FVC ratio was higher in HLA-DRB1\*15:01-positive patients ( $p = 9 \times 10^{-3}$ ).

**Conclusion:** We report an association among HLA-DRB1 alleles, COPD risk and pulmonary function parameters for the first time in Latin Americans. Since HLA-DRB1 genetic variability relates to the individual autoimmune response, these results support a role of autoimmunity in the pathogenesis of COPD.

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### Los alelos de HLA-DRB1 se asocian con la EPOC en una población latinoamericana mestiza

## RESUMEN

**Introducción:** Si bien los mecanismos moleculares de la patogénesis de la EPOC siguen sin ser claramente conocidos, cada vez existe más información que respalda que la autoinmunidad tiene un papel clave. Aunque los alelos de los antígenos leucocitarios humanos (HLA) se han asociado repetidamente con procesos autoinmunes, la relación entre los HLA y la EPOC permanece en gran parte inexplorada, especialmente en las poblaciones latinoamericanas (LA). En consecuencia, este estudio tuvo como objetivo investigar la presencia de los alelos de HLA de clase I y II en pacientes con EPOC y controles sanos en una población LA mestiza.

**Métodos:** Se analizó el genotipo de pacientes con EPOC ( $n = 214$ ) y controles de la misma edad ( $n = 193$ ) utilizando el Illumina Infinium Global Screening Array. Los alelos clásicos de los HLA se imputaron usando la imputación del genotipo HLA con empaquetamiento de atributos (HIBAG, por sus siglas en inglés) y el panel de referencia hispano. Finalmente, la distribución de los alelos HLA-DRB1 se reexaminó en 510 voluntarios no emparentados reclutados al azar.

## Palabras clave:

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**Resultados:** Los pacientes con EPOC mostraron una mayor frecuencia de alelos HLA-DRB1\*01:02 (6,54%) que los controles sanos (3,27%;  $p=0,04$ ; OR = 2,07). El HLA-DRB1\*01:02 también se asoció significativamente con el FEV<sub>1</sub> ( $p=0,04$ ) y la saturación de oxígeno ( $p=0,02$ ), y la relación FEV<sub>1</sub>/FVC fue mayor en los pacientes con HLA-DRB1\*15:01 ( $p=9 \times 10^{-3}$ ).

**Conclusión:** Comunicamos una asociación entre los alelos HLA-DRB1, el riesgo de la EPOC y los parámetros de la función pulmonar por primera vez en latinoamericanos. Dado que la variabilidad genética de HLA-DRB1 se relaciona con la respuesta autoinmune individual, estos resultados respaldan el papel de la autoinmunidad en la patogénesis de la EPOC.

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## Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide and it is expected to become the third by 2020.<sup>1</sup> In Latin American countries, the prevalence of COPD is 13.4% and its in-hospital mortality rate ranges from 6.7% to 29.5%.<sup>2</sup> In Chile, respiratory diseases are the third cause of death and, amongst them, COPD accounts for 22%, being the second cause of decease.<sup>3</sup>

COPD is a complex disease where genetic variants, environmental factors and random events interact to trigger pathological pathways. Genetic susceptibility is evident in familial clustering.<sup>4</sup> Specifically, population-based studies on families with respiratory disease have provided evidence for familial aggregation of spirometric parameters, and the heritability of lung function measures have been estimated to range from 10% to 80%.<sup>5,6</sup> Large genome-wide analysis studies (GWAS) have increased our knowledge about the genetic risk factors for lung function impairment and COPD.<sup>7</sup> However, most association studies trying to explain the genetic basis of COPD have been developed in Caucasian and Asian populations, showing some controversial results depending on the population analyzed.<sup>8</sup> So far, only four GWAS assessing lung function or COPD-related phenotypes have been focused on Hispanic/Latino populations, reporting both some novel loci (in or near the genes *KLHL7/NUPL2*, *DLG2*, *PDZD2* and *PRDM15*) and others previously identified in non-Hispanic samples.<sup>9–12</sup> Moreover, single nucleotide polymorphisms (SNPs) identified through GWAS only explain a small percentage of the heritability of lung function parameters, such as forced expiratory volume in one second (FEV<sub>1</sub>, 9.6%), forced vital capacity (FVC, 6.4%) and FEV<sub>1</sub>/FVC ratio (14.3%).<sup>13</sup>

Although the molecular mechanisms of COPD pathogenesis are still unclear, mounting evidence supports that autoimmunity could play a role.<sup>14,15</sup> Hence, the presence of autoimmunity-related responses elicited by T-helper type 1 (Th1) and Th17 cells have been reported in COPD patients,<sup>16,17</sup> whereas abnormal levels of pulmonary and circulating autoantibodies have also been associated to COPD.<sup>18,19</sup> Also, the results of some genetic studies have suggested a contribution of autoimmune responses to the development of COPD. For instance, Wain LV and colleagues have reported an association between decreased FEV<sub>1</sub> and the HLA-DQB1/HLA-DQA2 region.<sup>20</sup> In turn, a recent GWAS using samples from 18.335 Caucasian adults have identified a positive association of rs2074488, a SNP linked to the HLA-C gene, and COPD.<sup>21</sup> Since human leukocyte antigens (HLA) alleles have been repeatedly associated with auto-immune diseases,<sup>22,23</sup> these results reinforce the idea of an autoimmune component in COPD. Notwithstanding, the relation between HLA and COPD remains largely obscure, especially in Latin American (LA) populations.

To overcome this limitation, the present study aimed to analyze the presence of HLA class I and II alleles in COPD patients and healthy controls in a LA population with admixed ancestry.

## Methods

### Study population

We studied 214 COPD patients recruited at the Respiratory Service of the Hospital Regional de Talca, Chile, where they attended to undergo diagnostic tests after suspected COPD or for COPD monitoring visits. In parallel, 193 age-matched healthy controls were recruited at the same Hospital through a volunteer recruitment program. In order to rule out patients with asthma-COPD overlap syndrome, subjects with a history of asthma, rhinitis or any extra-pulmonary disease affecting lung function, and with positive bronchodilator test, FEV<sub>1</sub> increasing by  $\geq 12\%$  and 200 ml, were excluded from the study. In addition, 510 unrelated volunteers were randomly recruited among blood donors between January 2015 and May 2018 at Casa del Donante, Talca, Chile to reexamine the distribution of HLA-DRB1 alleles. 160 out of these 510 were submitted to HLA-A, -B and -C typing.<sup>24</sup>

Diagnostic evaluation of subjects was performed using GOLD criteria<sup>1</sup> and medical history was considered standardizing clinical information. Pulmonary function – including measurements of FEV<sub>1</sub>, FVC and carbon monoxide diffusing capacity of the lung (DL<sub>CO</sub>) – was assessed in all subjects using standard procedures<sup>25</sup> and equipment (Masterlab; Jaeger, Würzburg, Germany). Also, oxygen saturation was measured by pulse-oximetry (Ohmeda TuffSat, Soma Technology, Connecticut, USA). Body Mass Index (BMI) was calculated by dividing each person's weight in kilograms into their height in squared meters. Exercise capacity was determined with the distance walked in 6 minutes test (6MWT). The amount of cigarette smoking history was measured by pack-years and cumulative exposure to biomass smoke was calculated and expressed as hour-years as previously described.<sup>26</sup>

### Genotyping and imputation

Genotyping was performed using the Illumina Infinium Global Screening Array (Illumina, California, USA).<sup>12</sup> The classic HLA alleles at HLA-A, B, C, DPB1, DQA1, DQB1, and DRB1 were imputed using HLA Genotype Imputation with Attribute Bagging (HIBAG) with the Hispanic reference data set.<sup>27</sup>

### Statistical analyses

Clinic and demographical data were expressed as mean  $\pm$  standard deviation. Comparisons between COPD patients and subjects were performed using the Student's *t*-test. SNPs that met the quality criteria of a minor allele frequency (MAF) > 0.01, missingness < 0.1, and/or Hardy-Weinberg equilibrium (HWE)  $p > 0.001$  were included in the association analyses. A total of 7257 SNPs was located on chromosome 6 (chr6: 28400538–33489882, hg19) (genotyping rate: 0.93). For single-variant association

**Table 1**  
Demographic and clinical data.

	COPD patients n = 214	Control subjects n = 193
Sex, male/female	121/93	60/133
Age, years	70.97 ± 4.69	68.66 ± 3.25
Smoking history, pack-years	30.47 ± 14.82	7.75 ± 3.25*
Biomass exposure, hour-years	225.62 ± 54.28	96.87 ± 32.57*
FEV <sub>1</sub> , % predicted	61.47 ± 24.56	108.84 ± 18.40*
FEV <sub>1</sub> /FVC, %	58.25 ± 10.48	83.00 ± 6.27*
DL <sub>CO</sub> , % predicted	72.33 ± 25.13	87.43 ± 24.48*
Oxygen saturation, %	92.36 ± 4.76	96.14 ± 2.34*
BMI, kg/m <sup>2</sup>	26.96 ± 5.02	29.45 ± 5.02*
6 MW, meters	351.50 ± 155.61	462.95 ± 87.82*
mMRC	2.28 ± 1.39	–

Data presented as mean ± standard deviation, unless otherwise indicated. Definition of abbreviations: FEV<sub>1</sub>: forced expiratory volume in 1 second; FVC: forced vital capacity; DL<sub>CO</sub>: carbon monoxide diffusing capacity; BMI: body mass index; 6 MW: 6 minutes walking test; mMRC: modified Medical Research Council scale.

\* Different from COPD patients ( $p < 0.05$ ).

analysis, we used PLINK (v1.9) to perform logistic regression for binary phenotype (COPD and healthy controls),<sup>28</sup> HLA association analysis was performed with the PyHLA software<sup>29</sup> and R version 3.4.0. (<https://cran.r-project.org/>), using additive logistic regression models. Age, sex, and the first two principal components of a PCA based on genetic data, were used as covariates in all tests.

## Results

### Clinical and demographic findings

COPD patients and controls were similar with regard to age, while smoking pack-years and biomass exposure were significantly higher in COPD patients (Table 1). As expected, lung function parameters were significantly reduced in COPD patients, as well as oxygen saturation and 6MWT. Control subjects exhibited a significantly higher BMI than COPD patients and, interestingly, both groups showed overweight (BMI = 25.0 to <30).

### Genetic association between HLA-alleles and COPD

Of the 7257 SNPs genotyped, in the HLA region, ten markers showed suggestive associations ( $p < 5 \times 10^{-3}$ ) (Table 2, Supplementary Files 1). After HLA imputation, four HLA alleles showed a trend to association with COPD (Table 3;  $p < 0.1$ ). HLA-DRB1\*15:01 was decreased in patients with COPD compared to healthy controls, although no significant difference was observed (OR = 0.59, 95% CI 0.34–1.02). By contrast, the frequencies of HLA-B\*35:01 and HLA-C\*04:01 alleles were increased in COPD compared to controls (OR = 1.73, 95% CI 1.07–2.78; and OR = 1.49, 95% CI 1.01–2.21; respectively). The interaction test revealed that B\*35:01 and

C\*04:01 were in LD in both COPD and controls ( $p = 9.61 \times 10^{-36}$  and  $p = 5.67 \times 10^{-23}$ , respectively). Finally, we included in the analysis the HLA-A, -B, -C and -DRB1 typing performed in the Chilean population previously described by our group.<sup>24</sup> No differences were observed in the distribution of HLA-B\*35:01 and HLA-C\*04:01 alleles among control subjects and patients with COPD (Table 4). On the contrary, the HLA-DRB1\*01:02 allele frequency was increased in patients with COPD when compared with healthy controls (6.54% vs. 3.27%,  $p$  value < 0.05, OR = 2.07).

### Genetic association between HLA-alleles and clinical parameters

We also analyzed the possible influence of HLA class I and II alleles in pulmonary function, exercise capacity and oxygen saturation, in COPD patients (Table 5; Fig. 1). HLA-DRB1\*01:02 was significantly associated with FEV<sub>1</sub> and oxygen saturation ( $p = 0.04$  and  $p = 0.02$ ; respectively) (Fig. 1A and B). Moreover, FEV<sub>1</sub>/FVC ratio was higher among HLA-DRB1\*15:01-positive patients compared with HLA-DRB1\*15:01-negative patients ( $p = 9 \times 10^{-3}$ ) (Fig. 1C). Regarding exercise capacity, we observed that the median 6MWT value in the group of HLA-DRB1\*14:02-negative patients was higher as compared with the HLA-DRB1\*14:02-positive patient group ( $p = 0.04$ ) (Fig. 1D).

## Discussion

The present study aimed to investigate the association of HLA class I and II alleles and COPD in the Chilean population, characterized by a large admixture ancestry. We show for the first time that HLA-DRB1\*01:02 allele frequency is significantly increased in patients with COPD compared with healthy controls. Moreover, some HLA-DRB1 alleles correlated with clinical and pulmonary function parameters in COPD.

The HLA region plays a crucial role in numerous pathologies, as it accounts for 25% of known associations from the GWAS catalog (<https://www.ebi.ac.uk/gwas/>), especially with immune-related diseases. However, the relationship between HLA and COPD is unclear, since available data on this subject is scarce.<sup>30</sup> In this regard, a previous study reported that the presence of alanine at amino acid position 57 (instead of aspartic acid, valine or serine) in HLA-DQB1 was associated with decreased lung function.<sup>13</sup> In turn, Faner and co-workers reported a higher prevalence of DRB1\*14 in patients with severe airflow limitation and low DL<sub>CO</sub>.<sup>31</sup> Likewise, here we report associations of HLA-DRB1 alleles with pulmonary function (HLA-DRB1\*01:02 and HLA-DRB1\*15:01), exercise capacity (HLA-DRB1\*14:02) and oxygen saturation (HLA-DRB1\*01:02), in COPD patients. Indeed, the HLA-DRB1 is the most polymorphic gene within the HLA-II region, and it has been frequently associated with autoimmune diseases such as rheumatoid arthritis (RA), spondylarthritis, systemic lupus erythematosus and multiple sclerosis,

**Table 2**

HLA SNP association values based on the allele frequencies characterized for the COPD patients vs. healthy controls.

SNP	BP	Associated Gene	A1	MAFCOPD	MAFControls	p Value	OR (95% CI)
rs72881217	33119305	Intergenic	T	0.17	0.09	$9.43 \times 10^{-4}$	2.06 (1.33–3.18)
rs535586	31860337	EHMT2 (Synonymous Variant)	A	0.11	0.19	$1.04 \times 10^{-3}$	0.52 (0.35–0.77)
rs75456009	31321551	HLA-B (Downstream Variant)	G	0.31	0.21	$1.79 \times 10^{-3}$	1.66 (1.21–2.29)
rs521977	31836827	SLC44A4 (Intron Variant)	T	0.09	0.16	$2.67 \times 10^{-3}$	0.52 (0.34–0.80)
rs4713451	31272581	LOC107984146 (Intron Variant)	C	0.19	0.12	$3.35 \times 10^{-3}$	1.79 (1.21–2.65)
rs3130003	33331061	Intergenic	C	0.01	0.04	$3.43 \times 10^{-3}$	0.19 (0.05–0.65)
rs17206708	31384431	Intergenic	A	0.05	0.01	$3.58 \times 10^{-3}$	3.91 (1.46–10.48)
rs9267658	31845985	SLC44A4 (Intron Variant)	T	0.04	0.09	$3.96 \times 10^{-3}$	0.42 (0.23–0.77)
rs3130683	31888367	C2 (Intron Variant)	C	0.04	0.09	$4.72 \times 10^{-3}$	0.43 (0.24–0.78)
rs12661157	30986554	MUC22 (Intron Variant)	G	0.17	0.10	$4.86 \times 10^{-3}$	1.81 (1.79–2.75)

Only  $p$  values <  $5 \times 10^{-3}$  are shown. A1: minor allele nucleotide; COPD: chronic obstructive pulmonary disease; MAF: minor allele frequency; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

**Table 3**  
Frequency distribution of HLA alleles between COPD cases and controls.

HLA Allele	AF COPD cases	AF healthy controls	Padj	OR	95% CI
A*01:01	0.0958	0.0984	0.9001	0.97	0.62–1.53
A*02:01	0.2593	0.2358	0.4366	1.14	0.82–1.56
A*03:01	0.0678	0.0622	0.7437	1.10	0.62–1.94
A*11:01	0.0537	0.0492	0.7708	1.10	0.59–2.05
A*24:02	0.0818	0.0959	0.4859	0.84	0.52–1.36
A*26:01	0.0467	0.0570	0.5084	0.81	0.43–1.51
A*31:01	0.0748	0.0570	0.3127	1.33	0.76–2.34
A*68:01	0.1238	0.1192	0.8390	1.05	0.69–1.59
B*07:02	0.0467	0.0622	0.3300	0.74	0.40–1.36
B*08:01	0.0397	0.0622	0.1575	0.64	0.34–1.19
B*18:01	0.0514	0.0544	0.8479	0.94	0.51–1.74
B*35:01	0.1262	0.0777	0.0242	1.73	1.07–2.78
B*39:09	0.0981	0.0959	0.9105	1.03	0.64–1.66
B*44:03	0.0607	0.0544	0.6962	1.13	0.62–2.05
B*51:01	0.1121	0.0829	0.1457	1.44	0.88–2.35
C*04:01	0.1752	0.1244	0.0458	1.49	1.01–2.21
C*05:01	0.0537	0.0570	0.8419	0.94	0.52–1.70
C*06:02	0.0771	0.0803	0.8640	0.96	0.57–1.60
C*07:01	0.0794	0.1062	0.1860	0.72	0.45–1.17
C*07:02	0.1729	0.2228	0.0890	0.75	0.54–1.05
C*08:02	0.0491	0.0570	0.6036	0.85	0.45–1.59
C*15:02	0.0771	0.0725	0.7005	1.07	0.63–1.83
DQA1*01:01	0.0794	0.0725	0.7093	1.11	0.65–1.87
DQA1*01:02	0.1238	0.1269	0.8942	0.97	0.64–1.47
DQA1*02:01	0.1051	0.1114	0.7719	0.94	0.60–1.46
DQA1*03:01	0.1986	0.1865	0.6676	1.08	0.76–1.52
DQA1*04:01	0.0818	0.0803	0.9384	1.02	0.61–1.70
DQA1*05:01	0.0864	0.1010	0.4720	0.84	0.52–1.35
DQA1*05:03	0.0584	0.0415	0.2584	1.46	0.76–2.83
DQA1*05:05	0.1542	0.1632	0.7272	0.94	0.64–1.36
DQB1*02:01	0.0888	0.1010	0.5475	0.86	0.54–1.39
DQB1*02:02	0.1028	0.0959	0.7373	1.08	0.68–1.73
DQB1*03:01	0.2196	0.2202	0.9840	0.99	0.71–1.39
DQB1*03:02	0.2009	0.1865	0.6086	1.09	0.78–1.54
DQB1*04:02	0.0818	0.0803	0.9384	1.02	0.61–1.70
DQB1*05:01	0.0818	0.0777	0.8296	1.06	0.63–1.77
DQB1*06:02	0.0584	0.0907	0.0801	0.62	0.36–1.06
DQB1*06:03	0.0491	0.0518	0.8676	0.95	0.53–1.71
DRB1*03:01	0.0888	0.1010	0.5475	0.87	0.54–1.39
DRB1*04:07	0.1379	0.1399	0.9300	0.98	0.65–1.49
DRB1*07:01	0.1075	0.1114	0.8563	0.96	0.61–1.50
DRB1*08:02	0.0771	0.0751	0.9135	1.03	0.61–1.75
DRB1*11:01	0.0537	0.0544	0.9664	0.99	0.54–1.82
DRB1*14:02	0.0561	0.0466	0.5323	1.23	0.65–2.34
DRB1*15:01	0.0561	0.0907	0.0593	0.59	0.34–1.02

AF: allele frequency; Padj: *p*-values adjusted and corrected for FDR using the Benjamini–Hochberg test; OR: odds ratio; CI: confidence interval.

**Table 4**  
Frequency distribution of HLA alleles between COPD cases and controls.

HLA Allele	COPD cases ( <i>n</i> = 214)		Healthy controls ( <i>n</i> = 353)		<i>p</i> value	OR	95% CI
	<i>n</i>	%	<i>n</i>	%			
B*35:01	49	22.90	58	16.43	0.06	1.51	0.99–2.31
C*04:01	65	30.37	89	25.21	0.20	1.29	0.89–1.89

HLA Allele	COPD cases ( <i>n</i> = 214)		Healthy controls ( <i>n</i> = 703)		<i>p</i> value	OR	95% CI
	<i>n</i>	%	<i>n</i>	%			
DRB1*01:02	14	6.54	23	3.27	0.04	2.07	1.04–4.10
DRB1*14:02	23	10.75	48	6.83	0.08	1.64	0.97–2.77
DRB1*15:01	24	11.21	88	12.52	0.64	0.88	0.55–1.43

OR: odds ratio; CI: confidence interval.

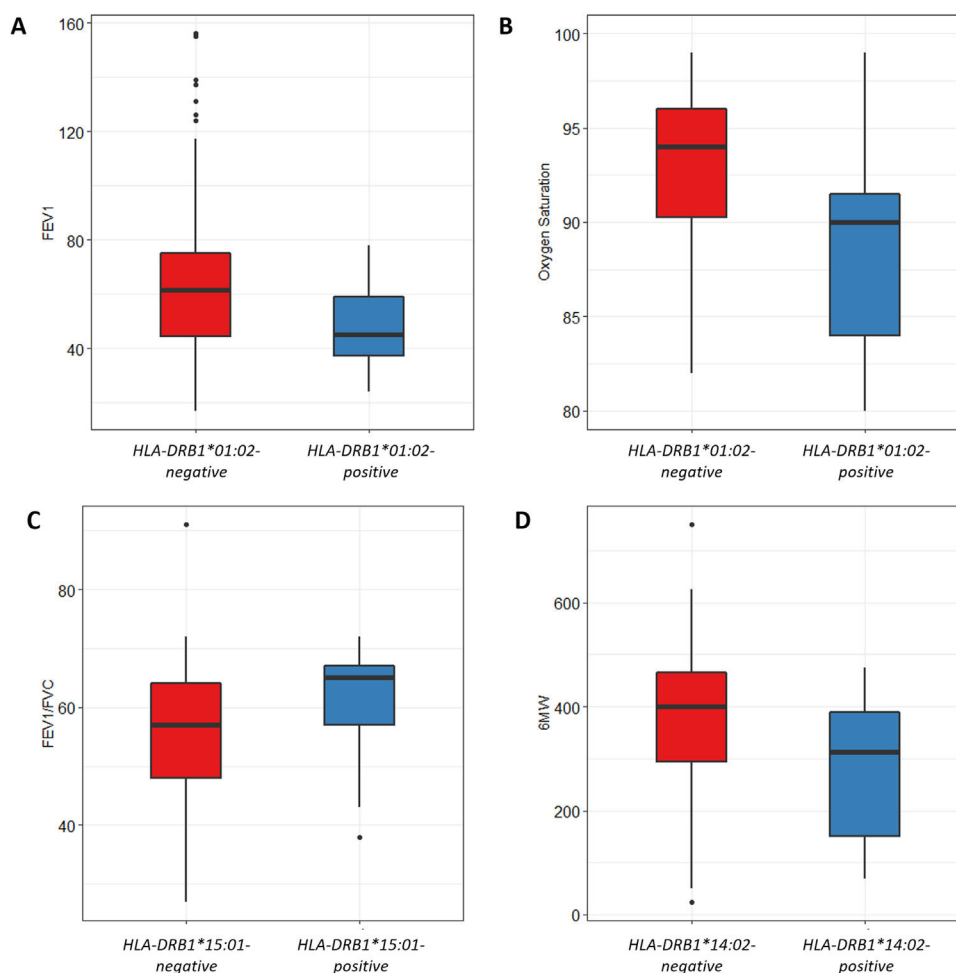
*p* values were determined by Fisher's 2-tailed exact test.

among others.<sup>32</sup> Moreover, a meta-analysis performed in Latin American patients with different autoimmune-diseases revealed that specific HLA-DRB1 polymorphisms are shared between more than one of these conditions.<sup>33</sup> Interestingly, it has been hypothesized that polymorphisms in HLA-II region could lead to the loss

of immunological tolerance to self-antigens.<sup>34</sup> In this regard, substantial evidence has shown the presence of self-antigens and autoantibodies in COPD patients.<sup>18,35,36</sup> Hence, the HLA-DRB1 alleles showing association with COPD risk and lung function parameters could be involved in this auto-immune mechanism.

**Table 5**  
Genetic association between HLA-alleles, exposure to COPD risk factors and clinical parameters.

	HLA-DRB1*01:02 Positive (n = 13)	HLA-DRB1*01:02 Negative (n = 201)	HLA-DRB1*14:02 Positive (n = 24)	HLA-DRB1*14:02 Negative (n = 190)	HLA-DRB1*15:01 Positive (n = 25)	HLA-DRB1*15:01 Negative (n = 189)
Smoking history, pack-years	41.95 ± 26.32	35.44 ± 49.07	26.67 ± 27.37	37.01 ± 49.93	31.42 ± 30.63	36.40 ± 49.79
Biomass exposure, hour-years	264.46 ± 198.46	249.74 ± 467.43	178.96 ± 232.40	259.64 ± 475.78	127.6 ± 141.26	266.82 ± 479.66
FEV <sub>1</sub> , % predicted	48.54 ± 15.03	62.77 ± 24.92*	55.71 ± 22.69	62.69 ± 24.82	66.04 ± 18.55	61.37 ± 25.33
FEV <sub>1</sub> /FVC, %	51.58 ± 12.45	56.45 ± 10.36	53.5 ± 10.68	56.46 ± 10.51	61.28 ± 9.14	55.45 ± 10.55**
DL <sub>CO</sub> , % predicted	68.85 ± 24.03	73.02 ± 25.25	68.09 ± 25.32	73.36 ± 25.12	75.71 ± 17.71	72.40 ± 25.88
Oxygen saturation, %	88.63 ± 6.37	92.48 ± 4.52*	91.77 ± 4.17	94.48 ± 4.84	94.77 ± 3.22	92.09 ± 4.84
BMI, kg/m <sup>2</sup>	26.91 ± 5.67	26.88 ± 5.04	27.57 ± 6.01	26.80 ± 4.94	27.69 ± 4.89	26.77 ± 5.08
6 MW, meters	398.57 ± 162.09	348.38 ± 154.73	266.58 ± 137.68	362.53 ± 154.22*	390.38 ± 150.85	346.44 ± 155.46

\*  $p < 0.05$ .\*\*  $p < 0.01$ .**Fig. 1.** Genetic association between HLA-alleles and clinical parameters in COPD cases. (A and B) HLA-DRB1\*01:02 was significantly associated with FEV<sub>1</sub> and oxygen saturation ( $p = 0.04$  and  $p = 0.02$ ; respectively). (C) HLA-DRB1\*15:01-positive patients exhibited a higher FEV<sub>1</sub>/FVC ratio than HLA-DRB1\*15:01-negative patients ( $p = 9 \times 10^{-3}$ ). (D) HLA-DRB1\*14:02-negative patients showed a higher 6MWT value ( $p = 0.04$ ).

It is noteworthy that HLA-DRB1 shared epitope (SE) alleles (which encode a common amino acid sequence), are the most important genetic contributors for the risk of developing anti-citrullinated protein autoantibodies (ACPA).<sup>37</sup> ACPA are different isotype autoantibodies that recognize the nonessential amino acid citrulline in proteins. Their presence has an important role in RA prognosis, since it is associated to severity of the disease.<sup>37</sup> In this regard, it has been suggested that lungs may be initiating sites of the generation of ACPA.<sup>38</sup> Although COPD is not a primary RA-related lung disease, a high prevalence of ACPA in COPD patients has been demonstrated.<sup>39</sup> Moreover, it has also been reported that heavy smokers with COPD are more

prone to ACPA production compared with heavy smokers without COPD.<sup>38</sup> Hence, higher ACPA levels could increase the risk to develop COPD, even in the absence of RA. Indeed, citrullinated proteins are increased in patients with COPD and correlate with ongoing inflammation.<sup>40</sup> Furthermore, it has been shown that anti-cyclic citrullinated peptide antibodies levels are higher in COPD patients exposed to biomass smoke.<sup>41</sup> Although ACPA determination was not performed in our cohort, COPD patients exhibited a substantial exposure to biomass smoke. In this frame, it is conceivably that HLA-DRB1 alleles could contribute to ACPA generation as a result of immunization to newly synthesized citrullinated peptides.

On the other hand, we acknowledge some limitations, being the relatively small sample size and limited statistical power the most important. Hence, the associations found between COPD risk and HLA-B\*35:01 and HLA-C\*04:01 alleles disappeared when study population was increased. Moreover, the imputation of HLA alleles in LA populations may be controversial due to the scant information available and the complexity of the admixture. The fact that the number of males in the control group almost doubled that of the patients group could have also biased our results, since biological and behavioral/environmental gender differences have been recognized to influence COPD development.<sup>42</sup> On the other hand, although we did not collect self-reported ethnicity, it has been shown that population from El Maule Region has a global ancestry estimate of 42.41% Native American, 55.62% European and 1.97% African.<sup>43</sup> This adds relevance to our analysis, since it is accepted that studies in admixed-ancestry populations could yield disease-associated loci that may have been missed due to allele frequency disparities.<sup>12</sup> Moreover, our findings are consistent with a plausible auto-immune process in COPD pathogenesis, which has been long recognized as a potential mechanism.

In conclusion, the present study shows an association among HLA-DRB1 alleles, COPD risk and COPD-related clinical parameters in an admixed LA population. Given that HLA-DRB1 gene is related to autoimmunity, our study supports the notion of an autoimmune process in the pathogenesis of COPD and justifies the need to develop more research on the association between HLA alleles and COPD risk.

#### Statement of ethics

This research complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

#### Authors' contributions

Study concept and design: JO, RDP; Data acquisition: RSS, SJ, JO; Data analysis: JO, RDP, HDH; Data interpretation: JO, RDP, MM; Funding acquisition: JO, RSS; Investigation: JO, RDP; Methodology: JO, RDP; Supervision: JO; Writing – original draft: JO, RDP; Writing – review & editing: JO, RDP, HDH, RSS, SJ, MM.

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#### Conflict of interest

Marc Miravittles has received speaker fees from AstraZeneca, Boehringer Ingelheim, Chiesi, Cipla, Menarini, Rovi, Bial, Sandoz, Zambon, CSL Behring, Grifols and Novartis, consulting fees from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Bial, Gebro Pharma, CSL Behring, Laboratorios Esteve, Ferrer, Mereo Bio-pharma, Verona Pharma, TEVA, pH Pharma, Novartis and Grifols and research grants from GlaxoSmithKline and Grifols, all outside the submitted work.

The rest of the authors declare no conflicts of interest.

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#### References

- (GOLD) Gifcold. 2020 Global strategy for prevention, diagnosis and management of COPD. 2020.
- Ciapponi A, Alison L, Agustina M, Demian G, Silvana C, Edgardo S. The epidemiology and burden of COPD in Latin America and the Caribbean: systematic review and meta-analysis. *COPD*. 2014;11:339–50.
- SZOT MJ. Mortalidad por enfermedades respiratorias en Chile durante 1999. *Revista chilena de enfermedades respiratorias*. 2003;19:8–14.
- Silverman EK, Chapman HA, Drazen JM, Weiss ST, Rosner B, Campbell EJ, et al. Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease Risk to relatives for airflow obstruction and chronic bronchitis. *Am J Respir Crit Care Med*. 1998;157:1770–8.
- Hallberg J, Iliadou A, Anderson M, de Verdier MG, Nihlen U, Dahlback M, et al. Genetic and environmental influence on lung function impairment in Swedish twins. *Respir Res*. 2010;11:92.
- Palmer LJ, Knuiman MW, Divitini ML, Burton PR, James AL, Bartholomew HC, et al. Familial aggregation and heritability of adult lung function: results from the Busselton Health Study. *Eur Respir J*. 2001;17:696–702.
- Hall R, Hall IP, Sayers I. Genetic risk factors for the development of pulmonary disease identified by genome-wide association. *Respirology*. 2019;24:204–14.
- Smolonska J, Wijmenga C, Postma DS, Boezen HM. Meta-analyses on suspected chronic obstructive pulmonary disease genes: a summary of 20 years' research. *Am J Respir Crit Care Med*. 2009;180:618–31.
- Manichaikul A, Hoffman EA, Smolonska J, Gao W, Cho MH, Baumhauer H, et al. Genome-wide study of percent emphysema on computed tomography in the general population. The Multi-Ethnic Study of Atherosclerosis Lung/SNP Health Association Resource Study. *Am J Respir Crit Care Med*. 2014;189:408–18.
- Chen W, Brehm JM, Manichaikul A, Cho MH, Boutaoui N, Yan Q, et al. A genome-wide association study of chronic obstructive pulmonary disease in Hispanics. *Ann Am Thorac Soc*. 2015;12:340–8.
- Burkart KM, Sofer T, London SJ, Manichaikul A, Hartwig FP, Yan Q, et al. A genome-wide association study in hispanics/latinos identifies novel signals for lung function. The Hispanic Community Health Study/Study of Latinos. *Am J Respir Crit Care Med*. 2018;198:208–19.
- Hosgood Iii HD, Diaz-Pena R, Blansky D, Jaime S, Parra V, Boekstegers F, et al. PRDM15 is associated with risk of chronic obstructive pulmonary disease in a rural population in Chile. *Respiration*. 2020:1–9.
- Wain LV, Shrine N, Artigas MS, Erzurumluoglu AM, Noyvert B, Bossini-Castillo L, et al. Genome-wide association analyses for lung function and chronic obstructive pulmonary disease identify new loci and potential druggable targets. *Nat Genet*. 2017;49:416–25.
- Kheradmand F, Shan M, Xu C, Corry DB. Autoimmunity in chronic obstructive pulmonary disease: clinical and experimental evidence. *Expert Rev Clin Immunol*. 2012;8:285–92.
- Caramori G, Ruggeri P, Di Stefano A, Mumby S, Girbino G, Adcock IM, et al. Autoimmunity and COPD: clinical implications. *Chest*. 2018;153:1424–31.
- Lee SH, Goswami S, Grudo A, Song LZ, Bandi V, Goodnight-White S, et al. Antielastin autoimmunity in tobacco smoking-induced emphysema. *Nat Med*. 2007;13:567–9.
- Shan M, Cheng HF, Song LZ, Roberts L, Green L, Hacken-Bitar J, et al. Lung myeloid dendritic cells coordinately induce TH1 and TH17 responses in human emphysema. *Sci Transl Med*. 2009;1, 4ra10.
- Feghali-Bostwick CA, Gadgil AS, Otterbein LE, Pilewski JM, Stoner MW, Cszimadia E, et al. Autoantibodies in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;177:156–63.
- Nunez B, Sauleda J, Anto JM, Julia MR, Orozco M, Monso E, et al. Anti-tissue antibodies are related to lung function in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2011;183:1025–31.
- Wain LV, Shrine N, Miller S, Jackson VE, Ntalla I, Soler Artigas M, et al. Novel insights into the genetics of smoking behaviour, lung function, and chronic obstructive pulmonary disease (UK BiLEVE): a genetic association study in UK Biobank. *Lancet Respir Med*. 2015;3:769–81.
- Ji X, Niu X, Qian J, Martucci V, Pendergrass SA, Gorlov IP, et al. A phenotype-wide association study uncovers a role for autoimmunity in the development of chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol*. 2018;58:777–9.
- Bodis G, Toth V, Schwarting A. Role of Human Leukocyte Antigens (HLA) in autoimmune diseases. *Rheumatol Ther*. 2018;5:5–20.
- Rotival M. Characterising the genetic basis of immune response variation to identify causal mechanisms underlying disease susceptibility. *Hla*. 2019;94:275–84.
- Castro-Santos P, Olloquequi J, Diaz-Pena R. HLA-A, B, C and DRB1 alleles in a Chilean population from Talca. *Hla*. 2020;95:200–3.
- Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26:319–38.
- Olloquequi J, Jaime S, Parra V, Cornejo-Cordova E, Valdivia G, Agusti A, et al. Comparative analysis of COPD associated with tobacco smoking, biomass smoke exposure or both. *Respir Res*. 2018;19:13.
- Zheng X, Shen J, Cox C, Wakefield JC, Ehm MG, Nelson MR, et al. HIBAG-HLA genotype imputation with attribute bagging. *Pharmacogenom J*. 2014;14:192–200.

28. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559–75.
29. Fan Y, Song YQ. PyHLA: tests for the association between HLA alleles and diseases. *BMC Bioinformatics.* 2017;18:90.
30. Kurche JS, Schwartz DA. Deciphering the genetics of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2019;199:4–5.
31. Faner R, Nunez B, Sauleda J, García-Aymerich J, Pons J, Crespi C, et al. HLA distribution in COPD patients. *COPD.* 2013;10:138–46.
32. Arango MT, Perricone C, Kivity S, Cipriano E, Ceccarelli F, Valesini G, et al. HLA-DRB1 the notorious gene in the mosaic of autoimmunity. *Immunol Res.* 2017;65:82–98.
33. Cruz-Tapias P, Perez-Fernandez OM, Rojas-Villarraga A, Rodriguez-Rodriguez A, Arango MT, Anaya JM. Shared HLA class II in six autoimmune diseases in Latin America: a meta-analysis. *Autoimmune Dis.* 2012;2012:569728.
34. Fernando MM, Stevens CR, Walsh EC, De Jager PL, Goyette P, Plenge RM, et al. Defining the role of the MHC in autoimmunity: a review and pooled analysis. *PLoS Genet.* 2008;4:e1000024.
35. Packard TA, Li QZ, Cosgrove GP, Bowler RP, Cambier JC. COPD is associated with production of autoantibodies to a broad spectrum of self-antigens, correlative with disease phenotype. *Immunol Res.* 2013;55:48–57.
36. Wells JM, Gaggar A, Nicod LP. A fragment in time. Elastin peptides and progression of emphysema. *Am J Respir Crit Care Med.* 2015;192:1141–3.
37. Castro-Santos P, Díaz-Peña R. Genetics of rheumatoid arthritis: a new boost is needed in Latin American populations. *Rev Bras Reumatol Engl Ed.* 2016;56:171–7.
38. Perry E, Kelly C, Eggleton P, De Soyza A, Hutchinson D. The lung in ACPA-positive rheumatoid arthritis: an initiating site of injury? *Rheumatology (Oxford).* 2014;53:1940–50.
39. Wood AM, de Pablo P, Buckley CD, Ahmad A, Stockley RA. Smoke exposure as a determinant of autoantibody titre in alpha(1)-antitrypsin deficiency and COPD. *Eur Respir J.* 2011;37:32–8.
40. Lugli EB, Correia RE, Fischer R, Lundberg K, Bracke KR, Montgomery AB, et al. Expression of citrulline and homocitrulline residues in the lungs of non-smokers and smokers: implications for autoimmunity in rheumatoid arthritis. *Arthritis Res Ther.* 2015;17:9.
41. Sigari N, Moghimi N, Shahraki FS, Mohammadi S, Roshani D. Anti-cyclic citrullinated peptide (CCP) antibody in patients with wood-smoke-induced chronic obstructive pulmonary disease (COPD) without rheumatoid arthritis. *Rheumatol Int.* 2015;35:85–91.
42. Aryal S, Diaz-Guzman E, Mannino DM. COPD and gender differences: an update. *Transl Res.* 2013;162:208–18.
43. Eyheramendy S, Martinez FI, Manevy F, Vial C, Repetto GM. Genetic structure characterization of Chileans reflects historical immigration patterns. *Nat Commun.* 2015;6:6472.