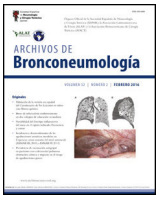
Archivos de Bronconeumología 58 (2022) T454–T456

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE Bronconeumología

www.archbronconeumol.org



Scientific Letter

[Translated article] Polymorphisms in the *FRMD4A* Gene Are Associated With Chronic Obstructive Pulmonary Disease Susceptibility in a Latin American Population

Polimorfismos en el gen FRMD4A se asocian a riesgo de enfermedad pulmonar obstructiva crónica en población latinoamericana

To the Director,

According to the estimates of the PLATINUM study, 16.9% of the population of Santiago de Chile aged 40 years or older have COPD,[1] while preliminary results from our MaulEPOC study showed that 90% of COPD patients in Chile's Maule region were smokers or former smokers.[2] One of the reasons for the high incidence of COPD in Chile could be the high prevalence of smoking in the population.[3]

Apart from alpha-1-antitrypsin deficiency, many other genetic variants associated with the disease have been described, including *CHRNA3/5*,[4] a region also associated with nicotine addiction. This suggests that other smoking-related genes may also play an important role in the pathogenesis of COPD.[5] In this respect, some single nucleotide polymorphisms (SNPs) in the *FRMD4A* gene have been described that are significantly associated with nicotine dependence in Asians,[6] some of which were also replicated in Caucasian and African-American populations. To date, however, the *FRMD4A* gene has not been studied in Latin American subjects with and without COPD. The aim of this paper, therefore, was to analyze the contribution of SNPs located in the *FRMD4A* gene to COPD susceptibility in the Chilean population.

A total of 568 subjects (322 with COPD and 246 healthy controls) were recruited from the Hospital Regional de Talca between 2016 and 2018. They all signed an informed consent form approved by the ethics committee of the Maule Health Service. A diagnosis of COPD was confirmed according to the GOLD criteria,[7] and clinically stable COPD patients and healthy controls underwent functional testing. A blood sample was also obtained for molecular testing and a standardized questionnaire[2] was completed on social and demographic history, current treatment, comorbidities, CAT index, dyspnea grade according to the modified Medical Research Council scale, number of exacerbations in the last 2 years, and number of exacerbations requiring hospitalization. History of exposure to known COPD risk factors was also recorded, including smoking history (pack-year index) and exposure to biomass smoke in the home. Our study complied with the guidelines for studies in humans and was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki.

Table 1
Clinical and epidemiological data.

	Controls $n = 193$	COPD $n = 214$
Sex, M/W, N (%)	60 (31)/133 (69)	121 (57)/93 (43)*
Age, years	68.66 ± 3.25	70.97 ± 4.69
Smoking history, pack-years	7.75 ± 3.25	30.47 ± 14.82*
Smoking habit, n (%)		
Smokers	31 (16)	29 (13)
Former smokers	63 (33)	136 (64)
Never smokers, %	99 (51)	49 (23)
Exposure to biomass, hours/year	96.87 ± 32.57	225.62 ± 54.28*
Schooling, years completed	14.33 ± 2.57	7.21 ± 3.98*
BMI, kg/m²	29.45 ± 5.02	26.96 ± 5.02*
Exacerbations in the previous year	–	1.37 ± 1.50
FEV_1, % predicted	108.84 ± 18.40	61.47 ± 24.56*
FEV_1/FVC,%	83.00 ± 6.27	58.25 ± 10.48*
DL_{CO}, % predicted	87.43 ± 24.48	72.33 ± 25.13*
Oxygen saturation, %	96.14 ± 2.34	92.36 ± 4.76*
6MWT, meters	462.95 ± 87.82	351.50 ± 155.61*
mMRC	–	2.28 ± 1.39
CAT	–	14.94 ± 8.46
BODE	–	3.18 ± 2.74

Data displayed as mean ± standard deviation, except where otherwise noted.
6MWT: 6-minute walk test; BMI: body mass index; BODE: *Body mass, airflow obstruction, dyspnea and exercise*; CAT: COPD Assessment Test; DLCO: diffusing capacity of the lung for carbon monoxide; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; mMRC: modified Medical Research Council dyspnea scale.
* Indicates a significant difference compared to controls ($p < 0.05$).

Genotyping was performed in 214 COPD patients and 193 healthy controls of the overall cohort. We selected 60 haplotype SNP markers (ht-SNP) based on linkage disequilibrium (LD) patterns located within the *FRMD4A* gene, using the HapMap dataset.[8] Haplotype SNP markers were selected with the Haploview Tagger tool,[9] according to the following criteria: low-frequency allele ≥ 0.01 and $r^2 > 0.8$, based on the HapMap populations (CEU = European reference population and MEX = Mexican native reference population). Genotyping was performed using the OpenArray®TM *TaqMan* platform (Applied Biosystems Inc., California, USA).

The sex variable was expressed by number of subjects, and differences between study groups were determined by the Chi-squared test. Continuous variables were expressed by mean and standard deviation. For the latter, an ANOVA variance analysis was performed, taking a p-value < 0.05 as significant. These analyses were conducted with the statistical R package (R Foundation for Statistical Computing, Vienna, Austria). Allele frequencies between COPD patients and healthy controls were compared using the Chi-squared test, and odd ratios with 95% confidence intervals were calculated using the PLINK program.[10]

DOI of original article: https://doi.org/10.1016/j.arbres.2022.01.016

https://doi.org/10.1016/j.arbres.2022.01.025

Table 2
Variants significantly associated with COPD susceptibility in the overall study cohort (all subjects) and in smokers only.

All subjects								
SNP	Position	Odds ratio	95% CI	*p*-Value	Bonferroni *p*-value	A1	MAF cases	MAF controls
rs10906545	13978235	1.97	1.47–2.62	3.97×10^{-6}	2.38×10^{-4}	A	0.54	0.37
rs1218353	14318441	2.41	1.54–3.77	7.82×10^{-5}	4.69×10^{-3}	G	0.18	0.08
Smokers only[a]								
SNP	Position	Odds ratio	95% CI	*p*-Value	Bonferroni *p*-value	A1	MAF cases	MAF controls
rs10906545	13978235	2.30	1.59–3.33	8.87×10^{-6}	5.32×10^{-4}	A	0.55	0.35
rs1534627	13969539	2.11	1.46–3.06	6.77×10^{-5}	4.06×10^{-3}	T	0.48	0.31

95% CI: 95% confidence interval; A1: low-frequency allele; MAF: minor allele frequency; SNP: single nucleotide polymorphism.
[a] 165 cases and 94 controls.

Significant differences were observed in the sex ratios between control subjects and COPD patients ($p < 0.05$), although ages were similar in both groups ($p > 0.05$; Table 1). COPD patients showed reduced FEV_1, FEV_1/FVC, DL_{CO}, and oxygen saturation values, as well as poorer performance in the 6-minute walk test ($p < 0.05$; Table 1). COPD patients also had a greater cumulative exposure to tobacco and biomass smoke ($p < 0.05$; Table 1).

The selected SNPs showed a low-frequency allele > 0.01, successful genotyping in at least 90% of the samples, and panmictic equilibrium, $p > 0.005$, so all were included in the association study. To compare the frequencies of the different SNP markers, the Bonferroni correction was applied by multiplying the *p*-value by the number of SNPs analyzed in the study ($n = 60$) to obtain the corrected *p*-value (Bonferroni *p*-value). *p*-Values below 0.05 were considered significant. Seven markers showed a tentative association with COPD susceptibility ($p < 0.05$), of which rs10906545 and rs1218353 showed the highest association (Bonferroni *p*-value < 0.05) (Table 2 [all subjects]). When only smokers were analyzed, both COPD patients and controls, rs10906545 and rs1534627 were significantly associated with COPD susceptibility (Table 2 [smokers only]). Since the intensity of smoking measured in pack-years was significantly higher in smokers with COPD than in smokers without COPD (36.77 ± 16.22 versus 14.25 ± 9.63, respectively, $p < 0.05$), the association may be more closely linked to nicotine dependence (and, as such, to the cumulative exposure to tobacco smoke) than to intrinsic pathogenic mechanisms activated by precipitating factors. In contrast, no association was detected when only patients and controls with no smoking history were analyzed (data not shown).

Our results show for the first time that the SNPs rs10906545, rs1218353, and rs1343005 are associated with an increased risk of developing COPD among smokers. Although Yoon et al. previously reported that the rs4424567 polymorphism in *FRMD4A* was associated with nicotine dependence,[6] it showed no association in our cohort or proximity to any of the SNPs that were significantly associated with COPD. Moreover, the rs1534627 SNP was only associated with COPD among smokers, and the association was lost when non-smokers were included, probably due to a statistical effect in which non-smokers offset the differences observed in smokers.

FRMD4A codes for a scaffolding protein that activates ADP-ribosylation factor 6 (Arf6)[11] that is involved in cell signal transduction, membrane traffic, and actin cytoskeleton organization.[12] *FRMD4A* gene overexpression has been associated with squamous cell carcinoma of the head and neck and non-small cell lung carcinoma.[13,14] *FRMD4A* hypermethylation has also been reported in MCF-7 cells treated with benzopyrene,[15] a polycyclic aromatic hydrocarbon present in many environmental pollutants, including biomass smoke and cigarette smoke.[16] In the case of COPD, variations have been described in the DNA methylation pattern between patients with COPD or reduced lung function and control subjects.[17,18] In this respect, a recent study by Morrow et al. revealed differences in *FRMD4A* methylation between subjects with and without COPD.[19] In addition, FRMD4A has also been reported to be associated with nicotine dependence,[14] and smoking during pregnancy has recently been shown to cause changes in the *FRMD4A* methylation pattern in newborns, and that these changes persist for many years after prenatal exposure.[20]

In conclusion, the present study is the first to describe an association between *FRMD4A* polymorphisms and the risk of developing COPD. Overall, our results and those of previous studies suggest that both genetic and epigenetic variations in the *FRMD4A* gene could be involved in the pathogenesis of COPD. Since *FRMD4A* is also associated with nicotine dependence, our results underscore the need to develop new strategies for smoking and COPD.

Funding

This study has been funded by the Chilean National Research and Development Agency (ANID, Fondecyt #11150022). Dr. Jordi Olloquequi received a grant from a Serra Húnter contract from the Generalitat de Catalunya (UB-LE-9035). Dr. Roberto Díaz-Peña received a grant from an ISCIII Miguel Servet contract (CP21/00003).

Acknowledgments

The authors thank study participants for their willingness to contribute to medical research, and Ms. Cintia Munoz and Ms. Hanuxa Celedón for their technical support in the research. We also thank Dr. Robert J.A. Halford for his intellectual contribution during the design of the MaulEPOC project.

References

1. Menezes AM, Perez-Padilla R, Hallal PC, Jardim JR, Muino A, Lopez MV, et al. Worldwide burden of COPD in high- and low-income countries. Part II. Burden of chronic obstructive lung disease in Latin America: the PLATINO study. Int J Tuberc Lung Dis. 2008;12:709–12.
2. Olloquequi GJ, Jaime JS, Parra RV, Muñoz VC, Muñoz GA, Lastra FF, et al. Caracterización general de los pacientes con EPOC de la Región del Maule: resultados preliminares del estudio MaulEPOC. Revista Chilena de enfermedades respiratorias. 2017;33:284–92.
3. (OPS) OPdIS. Informe sobre el control del tabaco en la Región de las Américas 2018. 2018.
4. Ragland MF, Benway CJ, Lutz SM, Bowler RP, Hecker J, Hokanson JE, et al. Genetic advances in COPD: insights from COPDGene. Am J Respir Crit Care Med. 2019.
5. Ishii T, Hagiwara K. Genetic predisposition to COPD: are there any relevant genes determining the susceptibility to smoking? In: Nakamura H, Aoshiba K, editors. Chronic obstructive pulmonary disease. Respiratory disease series: diagnostic tools and disease managements. Singapore: Springer; 2017. https://doi.org/10.1007/978-981-10-0839-9_3.
6. Yoon D, Kim YJ, Cui WY, Van der Vaart A, Cho YS, Lee JY, et al. Large-scale genome-wide association study of Asian population reveals genetic factors in FRMD4A and other loci influencing smoking initiation and nicotine dependence. Hum Genet. 2012;131:1009–21.
7. GOLD. Global Strategy for diagnosis, management, and prevention of COPD-2016; 2016.

8. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007;449:851–61.
9. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263–5.
10. 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.
11. Ikenouchi J, Umeda M. FRMD4A regulates epithelial polarity by connecting Arf6 activation with the PAR complex. Proc Natl Acad Sci USA. 2010;107:748–53.
12. Donaldson JG. Multiple roles for Arf6: sorting, structuring, and signaling at the plasma membrane. J Biol Chem. 2003;278:41573–6.
13. Goldie SJ, Mulder KW, Tan DW, Lyons SK, Sims AH, Watt FM. FRMD4A upregulation in human squamous cell carcinoma promotes tumor growth and metastasis and is associated with poor prognosis. Cancer Res. 2012;72:3424–36.
14. Velcheti V, Thawani R, Khunger M, Mukhopadhyay S, Chute DJ, Schrock AB, et al. FRMD4A/RET: a novel RET oncogenic fusion variant in non-small cell lung carcinoma. J Thorac Oncol. 2017;12:e15–6.
15. Sadikovic B, Andrews J, Rodenhiser DI. DNA methylation analysis using CpG microarrays is impaired in benzopyrene exposed cells. Toxicol Appl Pharmacol. 2007;225:300–9.
16. Silva R, Oyarzun M, Olloquequi J. Pathogenic mechanisms in chronic obstructive pulmonary disease due to biomass smoke exposure. Arch Bronconeumol. 2015;51:285–92.
17. Qiu W, Baccarelli A, Carey VJ, Boutaoui N, Bacherman H, Klanderman B, et al. Variable DNA methylation is associated with chronic obstructive pulmonary disease and lung function. Am J Respir Crit Care Med. 2012;185:373–81.
18. Machin M, Amaral AF, Wielscher M, Rezwan FI, Imboden M, Jarvelin MR, et al. Systematic review of lung function and COPD with peripheral blood DNA methylation in population based studies. BMC Pulm Med. 2017;17:54.
19. Morrow JD, Cho MH, Hersh CP, Pinto-Plata V, Celli B, Marchetti N, et al. DNA methylation profiling in human lung tissue identifies genes associated with COPD. Epigenetics. 2016;11:730–9.
20. Richmond RC, Suderman M, Langdon R, Relton CL, Davey Smith G. DNA methylation as a marker for prenatal smoke exposure in adults. Int J Epidemiol. 2018;47:1120–30.

Roberto Díaz-Peña[a,b], Raül F. Julià[c], Juan F. Montes[d], Rafael S. Silva[e], Jordi Olloquequi[f,b,*]

[a] *Fundación Pública Galega de Medicina Xenómica, SERGAS; Grupo de Medicina Xenomica-USC, Instituto de Investigación Sanitaria de Santiago (IDIS), Santiago de Compostela, Spain*
[b] *Laboratorio de Patología Celular y Molecular; Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile*
[c] *EDP Salut Sant Joan de Reus-Baix Camp; CAP La Selva del Camp, Tarragona, Spain*
[d] *Departament de Biologia Cel·lular, Fisiologia i Immunologia; Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain*
[e] *Unidad Respiratorio, Centro de Diagnóstico Terapéutico, Hospital Regional de Talca, Talca, Chile*
[f] *Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona, Spain*

Corresponding author.
E-mail address: jordiolloquequi@ub.edu (J. Olloquequi).