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Association between *LAG3/CD4* gene variants and risk of Parkinson's disease

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Abstract

Background/Objectives: Several recent studies suggest a possible role of lymphocyte activation 3 (LAG3) protein. LAG3 can behave as an α-synuclein ligand, and serum and cerebrospinal fluid-soluble LAG3 levels have been proposed as a marker of Parkinson's disease (PD). In this study, we aimed to investigate whether there is an association between 3 common single-nucleotide variations (SNVs) in the *LAG3* gene and its closely related *CD4 molecule* gene and the risk of PD in a Caucasian Spanish population. Two of them have been previously associated with the risk of PD in Chinese females.

Methods: We analysed genotypes and allele frequencies for *CD4* rs1922452, *CD4* 951818 and *LAG3* rs870849 SNVs, by using specifically designed TaqMan assays, in a cohort composed of 629 PD patients and 865 age- and gender-matched healthy controls.

Results: The frequencies of the *CD4* rs1922452 A/A genotype, according to the dominant and recessive genetic models, and of the *CD4* rs1922452/A allelic variant were significantly lower, and the frequencies of the *CD4* rs951818 A/A genotype, according to the dominant genetic model, and of the *CD4* rs951818/A allele, were significantly higher in PD patients than in controls. The differences were not significant after stratifying by sex. These two SNVs showed strong linkage. Regression models showed a lack of relation between the 3 SNVs studied and the age at onset of PD.

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Conclusions: These data suggest a possible role of *CD4* rs1922452 and *CD4* rs951818 polymorphisms in the risk of PD.

KEYWORDS

CD4 gene, genetics, LAG3 gene, Parkinson's disease, polymorphisms, risk factors

1 | INTRODUCTION

More than 200 years after the initial description of Parkinson's disease (PD), its aetiology has not been clearly established. Data from many studies reported during the last 35 years suggest that PD should be considered a genetically complex disease, with an interplay of both environmental and genetic factors. According to the Gene Database, there have been reported at least 24 genes related to susceptibility to familial PD, named *PARK1* to *PARK24* (link https://www.ncbi.nlm.nih.gov/gene, last accessed November 21, 2021), but these genes only explain a low percentage of the heritability of PD. Likely, meta-analyses from hypothesis-driven case–control association studies on candidate genes (many of them with variable and inconsistent results) and data from hypothesis-free Genome-Wide Association Studies (GWAS) could provide the most reliable data regarding the possible role of genetic polymorphisms in the risk of PD,[1] although a detailed revision of these studies is out of the scope of the present article.

One of the most important pathological hallmarks of PD is the abnormal expression and aggregation of α-synuclein (α-syn), which is the main protein component of Lewy bodies.[2] Lymphocyte activation 3 (LAG3) protein, which belongs to the immunoglobulin superfamily and is expressed by microglia, neurons and peripheral immune cells,[3] can bind to α-syn preformed fibrils with high affinity, and this binding initiates processes of endocytosis, transmission and toxicity induced by α-syn preformed fibrils leading to loss of dopamine neurons.[4] Some studies reported that the absence of LAG3 in neuronal cultures[4] and depletion of LAG3 in α-syn transgenic mice reduce aggregation of α-syn.[5] By contrast, another study did not find expression of LAG3 in human and murine neurons and did not find that overexpression of LAG3 in cultured human neural cells caused worsening of α-syn pathology, and did not confirm changes in survival in α-syn transgenic mice by LAG3 depletion.[6] Serum soluble LAG3 levels are significantly increased in PD patients compared with controls in two studies.[7,8] Another study showed similar serum soluble LAG3 levels but increased CSF-soluble LAG3 concentrations in PD patients compared with controls.[9] These data suggest that LAG3 could be a potential biomarker for PD, and its genetic variability can be related to the risk of presenting PD.

LAG3 protein is encoded by the *LAG3* gene (also known as *CD223* gene; chromosome 12p13.31; gene ID 3902, MIM 153337), which contains 8 exons and is closely related to the *CD4 molecule* gene, (*CD4*; gene ID 920, MIM 186940; this gene encodes the CD4 membrane glycoprotein of T lymphocytes, is expressed in several brain regions, acts as a coreceptor with the T-cell receptor to recognise antigens, participates in the early phase of T-cell activation and mediates indirect neuronal damage in immune-mediated and infectious diseases of the central nervous system). *LAG3* gene has been described recently as a possible predictor of the development of brain regional atrophy in PD patients.[10]

LAG3 (12:6881678-6887621) and *CD4* (12:6896024-6929974) are close genes. The main single nucleotide polymorphisms (SNVs) in *LAG3* and *CD4* genes are rs870849 T > C (chromosomal position 12:6887020, a missense variant located in the *LAG3* gene; its T allele has been related to protection against severity of primary immune thrombocytopenia),[11] rs1922452 A > G (chromosomal position 12:6896194, located in an intron within the *CD4* gene; it is associated with comorbidity in multiple sclerosis),[12] and rs951818 C > A (chromosomal position 12:6896055, located in a noncoding transcript exon in the *CD4* gene; it has been related to disease progression and mortality of sepsis).[13]

A recent case–control study, involving 646 PD patients and 536 healthy controls from a Chinese population, described significantly higher frequencies of *CD4* rs1922452/AA and *CD4* rs951818/CC genotypes in PD females than in their respective controls.[9] This study aims to replicate these findings in a large cohort of Caucasian Spanish PD patients and healthy controls.

2 | METHODS

2.1 | Study participants

The study involved 629 patients diagnosed with idiopathic PD (all of them aged more than 18 years) according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria,[14] all of them were recruited in the Movement Disorders Units from 4 Hospitals and examined personally by consultant neurologists specialised in

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this area, and 865 age- and sex-matched healthy controls. Recruitment of PD patients was done by 2 of these neurologists who served as Neurology consultants in 2 different hospitals (H.A-N and F.J.J-J), another who served as a Neurology consultant in other 2 of these hospitals (P.P), and another 2 in a single centre (M.B. and MA) through the inclusion period, being the inclusion criteria homogeneous. Controls were recruited from the University Hospital, Badajoz, Spain (404 subjects, most of them were students of staff from the University of Extremadura), and at the Clinica Universitaria de Navarra, Pamplona, Spain (461 subjects, healthy spouses of patients visiting this Hospital). All control individuals had no family history of PD, had no systemic or neurological diseases (including PD and other movement disorders) and underwent a medical examination prior to inclusion. Participants were recruited between January 2003 and March 2018. Table 1 summarises the demographic data of both PD patients and control subjects. These individuals have participated in a previous genetic association study.[15]

2.2 | Ethical aspects

The principles of the Helsinki Declaration were applied to the participants' recruitment, all of them signing their written informed consent after a full explanation of the study purpose and procedure. The study was approved by the Ethics Committees of Clinical Investigation of the Clínica Universitaria de Navarra (Pamplona, Spain), the Hospital Universitari Mutua de Terrassa (Terrassa, Barcelona, Spain) and the Infanta Cristina University Hospital (Badajoz, Spain).

2.3 | Genotyping of *LAG3* rs870849, rs1922452 and rs951818 variants

Genotyping was performed in genomic DNA obtained from peripheral leukocytes of venous blood samples of patients diagnosed with PD and controls. The analysis was performed by using real-time PCR (Applied Biosystems 7500 qPCR thermocycler) with specific custom-designed TaqMan probes (Life Technologies). Since this study aims to replicate previous findings, we selected the same SNVs analysed in the previous study. These SNVs include the only missense SNVs with an allele frequency over 0.01 in the population analysed and one intronic and one non-coding transcript exonic SNVs with high allele frequencies both, in the discovery population and in the population analysed here. The SNVs analysed and the TaqMan assays are rs870849 (C___9797874_10), rs1922452 (C__11914936_10) and rs951818 (C___8921385_10).

TABLE 1 Demographic data

Group	PD patients ($n = 629$)	Controls ($n = 865$)	P
Age, years, mean (SD)	66.65 (10.53)	65.22 (10.79)	.071
Age range, years	22–95	19–92	—
AAO, years, mean (SD)	57.68 (14.03)	NA	—
AAO range, years	14–85	NA	—
Men *n* (%)	295 (46.9)	392 (45.3)	.924

Abbreviations: AAO, age at onset; NA, not available; PD, Parkinson's disease; SD, standard deviation.

2.4 | Statistical analysis

We analysed the Hardy–Weinberg equilibrium by using the online application http://ihg.gsf.de/cgi-bin/hw/hwa1.pl, and the statistical package PLINK[16] was run to analyse the allele and the genotype frequencies. Finally, the SPSS 20.0 package (SPSS Inc.) was used to carry out the rest of the statistical analyses. χ^2 or, when appropriate, Fisher tests were used to perform intergroup comparisons and the false discovery rate procedure[17] to carry out the correction for multiple testing. Crude and corrected values (P and Pc, respectively) were obtained for each intergroup comparison, including all genotypes or all alleles. Haplotype analysis was carried out by using the SNPSTATS software (https://www.snpstats.net/).

To calculate the sample size, that was carried out based on the minor allele frequencies in control individuals, we analysed the frequency for carriers of the risk gene, using a relative risk (RR) value equal to 1.5 ($P < .05$).[18,19] The calculation of the statistical power for variant alleles in this study, based on the allele frequencies observed in healthy controls and in patients was, respectively, for one-tailed and two-tailed associations: rs870849 99.9% and 99.9%, rs1922452 99.9% and 99.9%, and rs951818 99.9% and 99.9%. The negative predictive values were calculated as described elsewhere.[20] Finally, a T-test for independent samples was used for the comparison of the age at PD onset across genotype categories for the 3 SNVs studied.

Reporting of the study conforms to broad EQUATOR guidelines,[21] specifically STROBE and STREGA checklists are summarised in Table S1.

3 | RESULTS

The genotypes of the 3 allelic variants studied were in Hardy–Weinberg's equilibrium, both in PD patients and controls. The P-values for the Hardy–Weinberg's equilibrium in the SNVs analysed were equal to 0.870, 0.940

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TABLE 2 Genotypes of patients with PD and healthy volunteers (control subjects) according to different genetic models

Variant	Genotype, *n* (%)			Codominant OR (95% CI)	*P*, Pc	Dominant OR (95% CI)	*P*, Pc	Recessive OR (95% CI)	*P*, Pc	Overdominant OR (95% CI)	*P*, Pc
rs1922452	AA	AG	GG								
Control	140 (16.2)	414 (47.9)	311 (36.0)								
PD	74 (11.8)	294 (46.7)	261 (41.5)	0.85 (0.68–1.06) 0.63 (0.45–0.87)	**.018, .054**	0.79 (0.64–0.98)	**.030, .045**	0.69 (0.51–0.93)	**.015, .045**	0.96 (0.78–1.17)	.670, .670
rs951818	AA	AC	CC								
Control	299 (34.6)	426 (49.2)	140 (16.2)								
PD	252 (40.1)	291 (46.3)	86 (13.7)	0.81 (0.65–1.01) 0.73 (0.53–1.00)	.075, .113	0.79 (0.64–0.98)	**.030, .045**	0.82 (0.61–1.10)	.180, .270	0.89 (0.72–1.09)	.250, .670
rs870849	CC	CT	TT								
Control	340 (39.3)	399 (46.1)	126 (14.6)								
PD	225 (35.8)	301 (47.9)	103 (16.4)	1.14 (0.91–1.43) 1.24 (0.91–1.68)	.330, .330	1.16 (0.94–1.44)	.160, .160	1.15 (0.87–1.52)	.340, .340	1.07 (0.87–1.32)	.510, .670

Abbreviation: PD, Parkinson's disease. Statistically significant values are marked in bold.

and 0.600 for the SNVs rs1922452 A/G, rs951818 A/C and rs870849 C/T, respectively. Table 2 shows the genotypes of PD patients and control subjects according to different genetic models. According to these results, the model providing the strongest predictive capacity is the dominant model. According to this model, the SNVs rs1922452 A/G and rs951818 A/C present statistically significant differences when comparing patients and control subjects. Such differences remain significant after false discovery rate (FDR) correction for multiple comparisons. The comparison of the allele frequencies, which is summarised in Table 3 indicated a statistically significant effect of the alleles rs1922452/G and rs951818/A, both being more frequent among patients with PD than in control individuals. The statistical significance is higher with the rs1922452/G variant but, for both SNVs, the differences remain significant after FDR correction for multiple comparisons.

We calculated the linkage disequilibrium between the three SNVs analysed. There is a strong linkage between the SNVs rs1922452 A/G and rs951818 A/C, with a D′ value equal to 0.950, an *r*-square value equal to 0.929 and a *P*-value equal to .0001. This is in agreement with the already described linkage for these two SNVs in the Iberian population in Spain, with D′ value equal to 1.000, *r*-square equal to 1.000 and *P*-value equal to .0001 according to the online tool LDpair from the National Cancer Institute (https://ldlink.nci.nih.gov/?tab=ldpair). In contrast, the SNV rs870849 C/T is not linked to the previously mentioned SNVs, with a D′ value equal to 0.313 and 0.34, an *r*-square value of .195 and −.194, and a *P*-value equal to .068 and .072 as compared to the SNVs rs1922452 A/G and rs951818 A/C, respectively. This is in agreement with the data obtained in LDpair for Iberian subjects: D′ equal to 0.231 and 0.231, *r*-square equal to .019 and .019, and *P*-value equal to .044 and .044, for the SNVs rs1922452 A/G and rs951818 A/C, respectively. Therefore our findings suggest that the two SNVs that are at linkage disequilibrium are associated with the risk of developing PD.

To refine the potential of the SNV genotyping analyses to identify the risk alleles, we analysed the putative role of the haplotypes with the risk and the results are summarised in Table 4. We identified 8 haplotypes in the population studied, but none of these had a strong association with the risk, thus suggesting that the putative effect of the SNVs is not accumulative.

Table 5 shows the genotypes of PD patients and control subjects, stratified by sex, according to different genetic models. The differences that were observed in the whole study group (Table 2) were not statistically significant when participants were stratified by sex. Neither were significant the allele frequencies as shown in Table 6. The trends towards the risk of genotypes and allele frequencies were similar to those reported for the

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TABLE 3 Allele frequencies patients with PD and healthy volunteers (control subjects)

Variant	Allele, *n* (%)		OR (95% CI)	*P*	Pc	NPV (95% CI)
rs1922452	A	G				
Control	694 (40.1)	1036 (59.9)				
PD	442 (35.1)	816 (64.9)	0.81 (0.70–0.94)	.006	.018	0.56 (0.55–0.57)
rs951818	A	C				
Control	1024 (59.2)	706 (40.8)				
PD	795 (63.2)	463 (36.8)	1.18 (1.02–1.38)	.027	.041	0.60 (0.58–0.63)
rs870849	C	T				
Control	1079 (62.4)	651 (37.6)				
PD	751 (59.7)	507 (40.3)	0.90 (0.77–1.04)	.139	.139	0.56 (0.54–0.59)

Note: Test for trend for rs1922452: OR = 1.25; chi-square = 7.71; $P = .005$. Test for trend for rs951818: OR = 0.85; chi-square = 4.94; $P = .026$.

Abbreviations: NPV, negative predictive value; *P*, crude probability; Pc, probability after multiple comparisons; PD, Parkinson's disease.

TABLE 4 Haplotype analysis

Haplotype (rs1922452 AG, rs951818 AC, rs870849 CT)	PD patients (%)	Controls (%)	OR (95% CI)	*P*
G A C	0.327	0.319	Reference	
G A T	0.296	0.265	1.08 (0.87–1.34)	.480
A C C	0.245	0.290	0.81 (0.65–1.01)	.066
A C T	0.097	0.097	0.95 (0.71–1.27)	.730
G C C	0.018	0.012	1.47 (0.76–2.83)	.250
Rare haplotypes	0.017	0.022	0.72 (0.41–1.26)	.250

Note: Rare haplotypes (combining any haplotypes with frequencies under 0.01) include the combinations GCT, AAC and AAT.

Abbreviation: PD, Parkinson's disease.

whole series, but the results were not statistically significant because of the sample size when patients were subdivided.

Age at onset of PD was similar for the 3 possible genotypes of *CD4* rs1922452, *CD4* rs951818 and *LAG3* rs870849 polymorphisms (Table 7). Regression models including sex and the age at onset were carried out. When the dependent variable was the risk of developing PD, three independent variables were significant: sex ($P < .001$), rs1922452 ($P = .005$) and rs951818 ($P = .036$). When the dependent variable was the age at onset, only sex ($P < .001$) was a significant factor. Although previous studies did not identify sex-related differences in the age at onset of PD,[22–24] in our cohort the age at onset is slightly higher in men (58.66 years, SD 11.89) than in women (56.28 years, SD 12.68); two-tailed *T*-test $P = .036$. This may be due to chance but would explain the finding suggesting that sex is a confounder in this particular study.

4 | DISCUSSION

Since the previously commented data suggest that LAG3 could be a reliable marker for PD,[7–9] it seems reasonable to address the possible association between the most relevant polymorphisms in the *LAG3* gene and in its closely related *CD4* gene with the risk of developing PD.

In this replication study, we found a modest risk increase for PD in carriers of the *CD4* rs951818/A allelic variant, while carriers of the *CD4* rs1922452/A had a modestly decreased risk of this disease. This result was not shown when analysing male and female individuals separately, a fact that should likely be related to an effect size. By contrast, we did not find an association between *LAG3* rs870849 SNV and the risk of PD.

It should be stated that the variant allele frequencies are similar in control individuals regarding the SNV rs870849/C in our study (0.624) and that described in East Asian individuals according to the gnomAD database (0.644). Also are similar the frequencies for rs951818/A (0.401 in our study and 0.352 in East Asians). In contrast, the frequencies for rs1922452/G are higher (0.599) in our study as compared with that of East Asians (0.352), thus increasing the chance of findings individuals with variant genotypes and therefore increasing the statistical power for this SNV in our study.

To our knowledge, only a previous study on Chinese subjects addressed the possible relationship between these polymorphisms and the risk of PD.[9] In such study, data on

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TABLE 5 Genotypes of patients with PD and healthy volunteers (control subjects), stratified by sex, according to different genetic models

Variant	Genotype, *n* (%)			Codominant OR (95% CI)	*P*, Pc	Dominant OR (95% CI)	*P*, Pc	Recessive OR (95% CI)	*P*, Pc	Overdominant OR (95% CI)	*P*, Pc
Women											
rs1922452	AA	AG	GG								
Control	79 (16.7)	225 (47.6)	169 (35.7)								
PD	42 (12.6)	153 (45.8)	139 (41.6)	0.87 (0.63–1.20) 0.61 (0.37–1.00)	.140, .300	0.81 (0.59–1.10)	.170, .210	0.66 (0.42–1.04)	.071, .213	0.98 (0.73–1.33)	.910, .910
rs951818	AA	AC	CC								
Control	168 (35.5)	229 (48.4)	76 (16.1)								
PD	134 (40.1)	155 (46.4)	45 (13.5)	0.77 (0.55–1.07) 0.71 (0.45–1.13)	.200, .300	0.75 (0.55–1.03)	.076, .210	0.83 (0.54–1.27)	.380, .430	0.85 (0.63–1.15)	.280, .795
rs870849	CC	CT	TT								
Control	185 (39.1)	221 (46.7)	67 (14.2)								
PD	122 (36.5)	160 (47.9)	52 (15.6)	1.19 (0.85–1.66) 1.30 (0.83–2.04)	.430, .430	1.22 (0.89–1.67)	.210, .210	1.18 (0.79–1.78)	.430, .430	1.10 (0.81–1.49)	.530, .795
Men											
rs1922452	AA	AG	GG								
Control	61 (15.6)	189 (48.2)	142 (36.2)								
PD	32 (10.8)	141 (47.8)	122 (41.4)	0.83 (0.61–1.12) 0.65 (0.42–1.00)	.120, .360	0.78 (0.58–1.04)	.090, .270	0.72 (0.48–1.07)	.100, .300	0.93 (0.70–1.23)	.620, .740
rs951818	AA	AC	CC								
Control	131 (33.4)	197 (50.3)	64 (16.3)								
PD	118 (40.0)	136 (46.1)	41 (13.9)	0.85 (0.63–1.15) 0.74 (0.48–1.14)	.340, .510	0.82 (0.62–1.10)	.180, .270	0.81 (0.55–1.21)	.310, .465	0.92 (0.70–1.22)	.570, .740
rs870849	CC	CT	TT								
Control	155 (39.5)	178 (45.4)	59 (15.1)								
PD	103 (34.9)	141 (47.8)	51 (17.3)	1.10 (0.81–1.49) 1.18 (0.77–1.81)	.720, .720	1.12 (0.84–1.49)	.460, .460	1.12 (0.75–1.66)	.580, .580	1.05 (0.79–1.39)	.740, .740

Abbreviation: PD, Parkinson's disease.

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TABLE 6 Alleles of patients with PD and healthy volunteers (control subjects) stratified by sex

Variant	Allele, *n* (%)		OR (95% CI)	*P*	Pc	NPV (95% CI)
Women						
rs1922452	A	G				
Control	383 (40.5)	563 (59.5)				
PD	237 (35.5)	431 (64.5)	0.81 (0.66–0.99)	.042	.126	0.57 (0.55–0.59)
rs951818	A	C				
Control	565 (59.7)	381 (40.3)				
PD	423 (63.3)	245 (36.7)	1.16 (0.95–1.43)	.144	.216	0.61 (0.58–0.64)
rs870849	C	T				
Control	591 (62.5)	355 (37.5)				
PD	404 (60.5)	264 (39.5)	0.92 (0.75–1.13)	.417	.417	0.57 (0.54–0.61)
Men						
rs1922452	A	G				
Control	311 (39.7)	473 (60.3)				
PD	205 (34.7)	385 (65.3)	0.81 (0.65–1.01)	.062	.137	0.55 (0.53–0.57)
rs951818	A	C				
Control	459 (58.5)	325 (41.5)				
PD	372 (63.1)	218 (36.9)	1.21 (0.97–1.51)	.091	.137	0.60 (0.57–0.63)
rs870849	C	T				
Control	488 (62.2)	296 (37.8)				
PD	347 (58.8)	243 (41.2)	0.87 (0.70–1.08)	.197	.197	0.55 (0.52–0.58)

Abbreviations: NPV, negative predictive value; *P*, crude probability; Pc, probability after multiple comparisons; PD, Parkinson's disease.

TABLE 7 Age at onset of PD according to the genotypes

	Age at onset (SD) years		
		Two-Tailed *T*-Test compared with A/A	**Two-Tailed *T*-Test compared with A/G**
rs1922452 AA	56.53 (12.68)		
rs1922452 AG	57.69 (11.97)	0.547	
rs1922452 GG	57.35 (12.25)	0.677	0.776
		Two-Tailed *T*-Test compared with A/A	**Two-Tailed *T*-Test compared with A/C**
rs951818 AA	57.31 (12.15)		
rs951818 AC	57.57 (12.29)	0.836	
rs951818 CC	56.45 (11.89)	0.633	0.537
		Two-Tailed *T*-Test compared with C/C	**Two-Tailed *T*-Test compared with C/T**
rs870849 CC	58.22 (11.35)		
rs870849 CT	56.74 (11.91)	0.220	
rs870849 TT	57.32 (14.21)	0.589	0.717

Abbreviation: PD, Parkinson's disease.

the whole series did not show an association between *CD4* rs1922452, *CD4* rs951818 and *LAG3* rs870849 and PD. However, in accordance with our findings, an association between *CD4* rs951818 and increased risk of PD (although in this study the association was restricted to females) was reported.[9] By contrast, our data on Caucasians suggested that *CD* rs1922452/A might be associated with decreased risk of developing PD. No information on such SNVs in

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the PD GWAS Locus Browser (https://pdgenetics.shinyapps.io/GWASBrowser/) was identified. This is expected since, although we identified statistically significant association in this study (Tables 2, 3 and 6) the strength of the association (*P*-values) are well below the threshold necessary to identify associations in GWAS.

The current study has several limitations that include the possibility of a selection bias (perhaps related to the fact that patient recruitment was done in a hospital setting), the relatively low sample size, despite our previous calculation of statistical power, which was adequate for odds-ratio (OR) detection of 1.5 and the lack of similar previous studies in Caucasians. These limitations warrant replication studies. In addition, it could not be excluded the possibility that some healthy controls subjects who participated in the study might develop PD in the future, but taking into account the PD incidence rates in subjects older than 65 in Spain[25] and the proportion of healthy controls carrying the risk genotype that might eventually develop PD, it is unlikely that this fact had a significant influence on the results of this study.

In summary, this study suggests a weak, although statistically significant, association between *CD4* rs1922452 and *CD4* rs951818 polymorphisms and the risk of PD in the Caucasian Spanish population.

AUTHORS' CONTRIBUTIONS

All authors fulfil the criteria of authorship, and no one else who fulfils the criteria has been excluded. All of them have approved the final submitted version. EGM involved in drafting/revising the manuscript for content, including medical writing for content, study concept or design, acquisition of data, interpretation of data, study supervision and coordination, and obtaining funding. PP involved in drafting/revising the manuscript for content, including medical writing for content, study concept or design, acquisition of data and interpretation of data. JGT involved in drafting/revising the manuscript for content, including medical writing for content and acquisition of data. HAN involved in drafting/revising the manuscript for content, including medical writing for content, study concept or design, acquisition of data, interpretation of data, study supervision and coordination. IA involved in drafting/revising the manuscript for content, including medical writing for content and acquisition of data. MB involved in drafting/revising the manuscript for content, including medical writing for content and acquisition of data. MOCA involved in drafting/revising the manuscript for content, including medical writing for content and acquisition of data. MA involved in drafting/revising the manuscript for content, including medical writing for content and acquisition of data. JAGA involved in drafting/revising the manuscript for content, including medical writing for content, study concept or design, acquisition of data, statistical analysis and interpretation of data, study supervision and coordination, and obtaining funding. FJJJ involved in drafting/revising the manuscript for content, including medical writing for content, study concept or design, acquisition of data, analysis or interpretation of data, study supervision and coordination.

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CONFLICT OF INTEREST

All authors declare that there is no financial or nonfinancial conflict of interest.

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