Letters to the Editors

LILRA3 deficiency is not involved in the giant cell arteritis and systemic sclerosis predisposition

Sirs,

Recently, a 6.7 kb genetic deletion of *LILRA3*, a member of the leukocyte immunoglobulin-like receptors family, was found to influence the genetic predisposition to different autoimmune conditions, such as rheumatoid arthritis (RA) (1), multiple sclerosis (MS) (2), Sjögren's syndrome (SS) (3, 4), and systemic lupus erythematosus (SLE) (4). This deletion comprises the first seven exons of the gene and leads to a non-functional protein due to the absence of the Ig-like domains of the receptor (5).

Identifying potential causal variants shared among related diseases would contribute to increase our understanding on the pathogenic pathways influencing autoimmune conditions. The aim of the present study was therefore to assess whether this deletion represents a novel genetic risk factor for two immune-mediated diseases, giant cell arteritis (GCA) and systemic sclerosis (SSc).

For this purpose, the *LILRA3* deletion was genotyped by polymerase chain reaction in a total of 1000 biopsy-proven GCA patients, 2013 SSc patients and 1978 healthy controls of Spanish origin. Specific primers for detecting presence (5'-GGCTCTGTGATCACCCAA-3' and 5'-CAGTGTGGCTGCCATAGAT-3') or absence (5'-CATCTCGATCTGCCACTGACAC-3' and 5'-GACAGCAGATTCTAAAACAGTGG-3') of the complete gene were used. Patients with GCA were stratified according to the main clinical complications of the disease, polymyalgia rheumatica, jaw claudication, visual manifestations and stroke. SSc subsets were established based on the extent of skin involvement and autoantibody status as limited or diffuse cutaneous SSc, positive for anticentromere or antitopoisomerase antibodies, as well as for the presence of pulmonary fibrosis. Since it has been described a sex-specific effect of the deletion in RA (1), stratification of patients by sex was also performed. Approval from the local ethical committees and informed written consent from all participants was obtained in accordance with the tenets of the Declaration of Helsinki.

Statistical power was calculated using CaTS (http://www.sph.umich.edu/csg/abecasis/CaTS/). Plink (v1.07) (http://pngu.mgh.harvard.edu/purcell/plink/) was used to perform chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were obtained according to Woolf's method. P-values lower than 0.05 were considered statistically significant.

No evidence of departure from Hardy-Weinberg equilibrium was observed (p>0.05). First, we analysed the possible implication of the *LILRA3* deletion in GCA and SSc by comparing allelic distributions of both case sets with controls. As shown in Table 1, no statistically significant association with any of these diseases was found (GCA: p-value=0.722, OR(CI 95%)=1.03 (0.89-1.19); SSc: p-value=0.346, OR(CI 95%)=0.94 (0.84-1.07)). Likewise, the recessive genetic test showed no significant effect of the homozygous deletion in GCA (p-value=0.826, OR(CI 95%)=0.94 (0.57-1.58)) or SSc (p-value=0.843, OR(CI 95%)=0.96 (0.63-1.45)). Stratified analyses by sex and according to the main clinical manifestations of each disease yielded similar negative results (data not shown).

The present data show a lack of association of the *LILRA3* deletion with GCA and SSc. Our study had a high statistical power to detect a similar effect to that reported for other autoimmune diseases (~100% to detect ORs previously described for MS (1.93), RA (1.92), SS (2.65) or SLE (2.03)) (1-4), therefore, it is unlikely that the lack of association observed herein was due to a type II error. According to our results, a very recent well-powered meta-analysis (6) has failed to confirm the association between the *LILRA3* deletion and MS previously described.

It is possible that other *LILRA3* polymorphisms, showing low linkage disequilibrium with that analysed in our study, influence these pathologies. Interestingly, in a recent GWAS (7), a SNP within *LILRA3* showed a suggestive association with Takayasu's arteritis, a large-vessel vasculitis similar to GCA. However, considering our results and the fact that no signals in this region were detected in previous large-scale genetic studies (8, 9), a relevant role of *LILRA3* in the GCA and SSc pathogenesis could be discarded.

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References

1. DU Y, CUI Y, LIU X *et al*.: Contribution of functional LILRA3, but not nonfunctional LILRA3, to sex bias in susceptibility and severity of anti-citrullinated protein antibody-positive rheumatoid arthritis. *Arthritis Rheumatol* 2014; 66: 822-30.
2. KOCH S, GOEDDE R, NIGMATOVA V *et al*.: Association of multiple sclerosis with ILT6 deficiency. *Genes Immun* 2005; 6: 445-7.
3. KABALAK G, DOBBERSTEIN SB, MATTHIAS T *et al*.: Association of immunoglobulin-like transcript 6 deficiency with Sjögren's syndrome. *Arthritis Rheum* 2009; 60: 2923-5.
4. DU Y, SU Y, HE J *et al*.: Impact of the leucocyte immunoglobulin-like receptor A3 (LILRA3) on susceptibility and subphenotypes of systemic lupus erythematosus and Sjögren's syndrome. *Ann Rheum Dis* 2015; 74: 2070-5.
5. TORKAR M, HAUDE A, MILNE S, BECK S, TROWSDALE J, WILSON MJ: Arrangement of the ILT gene cluster: a common null allele of the ILT6

Table I. Genotype and allele frequencies of the LILRA3 deletion in SSc and GCA patients and healthy controls.

	Genotype, n (%)				Allele test	
Subgroup (n)	+/+	±	-/-	MAF (%)	p-value*	OR [95% CI]**
Controls (n=1957)	1362 (69.60)	548 (28.00)	47 (2.40)	16.40		
GCA (n=969)	666 (68.73)	281 (29.00)	22 (2.27)	16.77	0.722	1.03 [0.89-1.19]
SSc (n=1905)	1354 (71.08)	507 (26.61)	44 (2.31)	15.62	0.346	0.94 [0.84-1.07]

*p-values for the allelic model. **Odds ratio for the minor allele. GCA: giant cell arteritis; SSc: systemic sclerosis; MAF: minor allele frequency; CI, confidence interval.

gene results from a 6.7-kbp deletion. *Eur J Immunol* 2000; 30: 3655-62.
6. ORTIZ MA, NUNEZ C, ORDONEZ D *et al*.: Influence of the LILRA3 Deletion on Multiple Sclerosis Risk: Original Data and Meta-Analysis. *PLoS One* 2015; 10: e0134414.
7. RENAUER PA, SARUHAN-DIRESKENELI G, COIT P *et al*.: Identification of Susceptibility Loci in IL6, RPS9/LILRB3, and an Intergenic Locus on Chromosome 21q22 in Takayasu Arteritis in a Genome-Wide Association Study. *Arthritis Rheumatol* 2015; 67: 1361-8.
8. BOSSINI-CASTILLO L, LOPEZ-ISAC E, MARTIN J: Immunogenetics of systemic sclerosis: Defining heritability, functional variants and shared-autoimmunity pathways. *J Autoimmun* 2015; 64: 53-65.
9. CARMONA FD, MACKIE SL, MARTIN JE *et al*.: A large-scale genetic analysis reveals a strong contribution of the HLA class II region to giant cell arteritis susceptibility. *Am J Hum Genet* 2015; 96: 565-80.

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