Evidence of association of the *NLRP1* gene with giant cell arteritis

Recent studies have focused attention on the involvement of *NLRP1* to confer susceptibility for extended autoimmune/inflammatory disorders, being considered a common risk factor in autoimmunity.[1–3] *NLRP1* provides a scaffold for the assembly of the inflammasome that activates caspases 1 and 5, required for processing and activation of the proinflammatory cytokines interleukin 1β (IL-1β), IL-18 and IL-33 and promoting inflammation.[4] In this study, we examined for the first time whether *NLRP1* is associated with giant cell arteritis (GCA), a chronic systemic vasculitis affecting large and medium-sized arteries derived from the aorta, in particular the cranial branches of the carotid artery. GCA is the most common vasculitis in the elderly in Western countries with a female predominance.[5] To investigate the possible genetic association of *NLRP1* with this disease, we genotyped a single-nucleotide polymorphism (rs8182352), which has been reported to confer risk to the development of autoimmune processes in previous studies,[1 2] in a total of 3583 individuals, comprising a discovery set from Spain (574 patients diagnosed with biopsy-proven GCA and 2366 healthy controls) and a replication set of subjects from Italy (111 biopsy-proven GCA patients and 532 controls) using a predesigned TaqMan allele discrimination assay. All individuals were of European Caucasian origin. Patients were stratified according to the presence or absence of polymyalgia rheumatica, visual ischaemic manifestations and irreversible occlusive disease, as previously described.[6 7] Approval from the local ethical committees and informed written consent from all participants were obtained. The analysed genetic variant rs8182352 showed statistically significant differences between GCA patients and unaffected

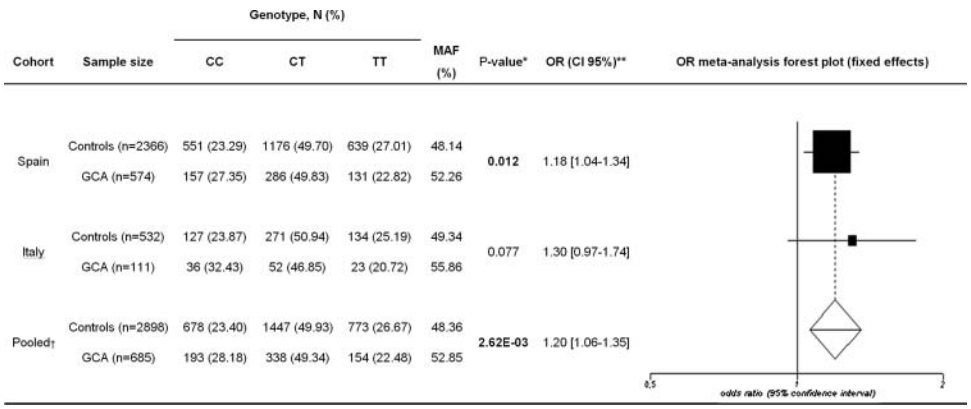

Figure 1 Genotype distribution and minor allele frequency (MAF) of the *NLRP1* polymorphism rs8182352 in giant cell arteritis (GCA) patients and healthy controls from Caucasian Spanish and Italian populations. The overall statistical power of the analysis was 88% to detect associations with OR=1.2 at the 5% significant level, according to Power Calculator for Genetic Studies 2006 software (http://www.sph.umich.edu/csg/abecasis/CaTS/). After genotyping, no evidence of departure from Hardy–Weinberg equilibrium was observed in either case or control populations (p=0.05). All statistical analyses of allele frequencies were performed using Plink V.1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). To test for associations, p values were obtained by performing 2×2 contingency tables and χ^2 test and/or Fisher's exact test, when appropriate. ORs and 95% CI were calculated according to Woolf's method. p Values lower than 0.05 were considered as statistically significant. Combined OR was calculated according to a fixed-effects model (Mantel–Haenszel meta-analysis) and the heterogeneity of the ORs among all populations was calculated using the Breslow–Day test. The forest plot was performed with StatsDirect V.2.4.6 (Altrincham, UK). *p Value for the allelic model. **OR for the minor allele.

controls in the Spanish cohort (p=0.012, OR=1.18, CI 95% =1.04 to 1.34) and in the combined meta-analysis ($p=2.62\times10^{-3}$, OR=1.20, CI 95%=1.06 to 1.35) including the Italian cohort (figure 1). Moreover, homozygous carriers of the allele C+ allele C (CC) genotype evidenced increased GCA risk in the combined data in comparison with non-CC carriers ($p=9.6\times10^{-3}$, OR=1.29, CI 95%=1.07 to 1.55). Despite their different geographical origin, we previously reported no differences in the clinical spectrum of the disease between both populations,[8] and the combinability test according to Breslow–Day method showed no significant heterogeneity in the ORs. When cases were meta-analysed against controls according to the specific clinical features of GCA, only a significant association with visual ischaemic manifestations was observed (p=0.014, OR=1.29, CI 95%=1.05 to 1.58). In addition, no statistically significant differences were observed when the subphenotype analysis was performed comparing GCA patients positive against those negative for the analysed clinical characteristic (data not shown).

This study shows for the first time that *NLRP1* rs8182352, a polymorphism with no predicted functional role, is associated with GCA in two different European populations. It has been proposed that *NLRP1* is an important regulator of different inflammatory and autoimmunity processes, mainly because of its ability to form the inflammasome that plays a pivotal role in the activation of IL-18 and IL-33.[4] Previous studies evidenced that IL-18, which is expressed by a wide range of immune cells and can mediate both Th1 and Th2 driven immune responses, contributes to the pathogenesis of GCA.[9] Furthermore, this vasculitis is associated with increased inflammatory response,[6] and IL-33 has been characterised as a critical component of the inflammatory disease, with immune cell activation.[10]

In conclusion, we have identified *NLRP1* as a novel GCA susceptibility gene, thus adding another piece to the genetic puzzle underlying the pathogenesis of this complex disease. Our data suggest that the inflammasome may represent a potential target for future therapeutic intervention. However, further studies may be performed to elucidate the possible causal variant/s of this association and its functional consequence, which is a limitation of this study.

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