RESEARCH ARTICLE

Protective Role of the Interleukin 33 rs3939286 Gene Polymorphism in the Development of Subclinical Atherosclerosis in Rheumatoid Arthritis Patients

Raquel López-Mejías1, Fernanda Genre1, Sara Remuzgo-Martínez1, Montserrat Robustillo-Villarino2, Mercedes García-Bermúdez3, Javier Llorca4, Alfonso Corrales1, Carlos González-Juanatey4, Begoña Ubilla1, José A. Miranda-Filloy6, Verónica Mijares1, Trinitario Pina1, Ricardo Blanco1, Juan J. Alegre-Sancho2, Marco A. Ramírez Huaranga4, María D. Mínguez Sánchez3, Beatriz Tejera Segura4, Iván Ferraz-Amaro5, Esther Vicente9, F. David Carmona1, Santos Castañeda9, Javier Martín3, Miguel A. González-Gay1,10,11

1 Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases, IDIVAL, Santander, Spain, 2 Rheumatology Department, Hospital Universitario Doctor Peset, Valencia, Spain, 3 Institute of Parasitology and Biomedicine López-Neyra, IPBLN-CSIC, Granada, Spain, 4 Department of Epidemiology and Computational Biology, School of Medicine, University of Cantabria, and CIBER Epidemiología y Salud Pública (CIBERESP), IDIVAL, Santander, Spain, 5 Cardiology Department, Hospital Lúcas Augusti, Lugo, Spain, 6 Rheumatology Department, Hospital Lúcas Augusti, Lugo, Spain, 7 Rheumatology Department, Hospital General Universitario de Ciudad Real, Ciudad Real, Spain, 8 Rheumatology Department, Hospital Universitario de Canarias, Tenerife, Spain, 9 Rheumatology Department, Hospital Universitario la Princesa, IIS-Princesa, Madrid, Spain, 10 Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, 11 Health Research Institute of Santiago de Compostela (IDIS), Division of Rheumatology, Clinical University Hospital of Santiago de Compostela, Santiago de Compostela, Spain

☯ These authors contributed equally to this work.
‡ MAGG and JM are joint senior authors on this work.
* miguelaggay@hotmail.com

Abstract

Objectives
To determine whether the interleukin-33 (IL-33)-interleukin-1 receptor like 1 (IL-1RL1) signaling pathway is implicated in the risk of subclinical atherosclerosis in patients with rheumatoid arthritis (RA).

Methods
A total of 576 Spanish RA patients from Northern Spain were genotyped for 6 well-known IL33-IL1RL1 polymorphisms (IL33 rs3939286, IL33 rs7025417, IL33 rs7044343, IL1RL1 rs2058660, IL1RL1 rs2310173 and IL1RL1 rs13015714) by TaqMan genotyping assay. The presence of subclinical atherosclerosis was determined by the assessment of carotid intima-media thickness (cIMT) by carotid ultrasound (US).
Results

RA patients carrying the TT genotype of the IL33 rs3939286 polymorphism had lower cIMT values than those homozygous for the CC genotype (mean ± standard deviation (SD): 0.71 ± 0.14 mm versus 0.76 ± 0.16 mm, respectively) while patients carrying the CT genotype had intermediate cIMT values (mean ± SD: 0.73 ± 0.17 mm). Moreover, RA patients carrying the mutant allele T of the IL33 rs3939286 polymorphism exhibited significantly lower cIMT values than those carrying the wild allele C (mean ± SD: 0.72 ± 0.16 mm versus 0.75 ± 0.18 mm respectively; p = 0.04). The association of both genotype and allele frequencies of IL33 rs3939286 and cIMT levels remained statistically significant after adjustment for sex, age at the time of US study, follow-up and center (p = 0.006 and p = 0.0023, respectively), evidencing that the potential effect conferred by IL33 rs3939286 may be independent of confounder factors. No association with other IL33-IL1RL1 genetic variants was observed.

Conclusions

In conclusion, our results may suggest a potential protective effect of the IL33 rs3939286 allele T in the risk of subclinical atherosclerosis in patients with RA.

Introduction

Rheumatoid arthritis (RA) is a complex autoimmune disease associated with progressive disability, systemic complications and early death [1]. Mortality is higher among RA patients than in the general population, and cardiovascular (CV) complications remain a major challenge [2]. The mechanisms leading to accelerated atherosclerosis in RA are complex, including not only the effect of traditional CV risk factors and chronic inflammation [2–3]. In this regard, several pieces of evidence indicate that genetic polymorphisms located inside and outside of the human leukocyte antigen (HLA) region also influence the risk of CV disease in RA [2,4–5]. Subclinical atherosclerosis has been observed in RA patients, even in those without traditional CV risk factors, and abnormally high values of carotid intima-media thickness (cIMT) have been found to predict the risk of CV events in these patients [6].

Interleukin-33 (IL-33) is a newly characterized cytokine that belongs to the IL-1 family [7]. This cytokine is constitutively expressed by tissue barrier cells (such as the epithelial and endothelial cells of many organs), but it is also expressed by some innate immune cells (such as macrophages and dendritic cells). IL-33 displays both nuclear and extracellular effects and mediates its biological function by interacting with its receptor (IL-1 receptor like 1 (IL-1RL1) also called ST2) and coreceptor (IL-1 receptor accessory protein (IL-1RAcP)). Since this binding exerts relevant immunomodulatory functions producing chemokines and pro-inflammatory and Th2-associated cytokines [7], IL-33-IL-1RL1 has been considered as a key pathway involved in inflammatory diseases. In accordance with that, a pathogenic role of IL-33 has been found in RA, where its levels are significantly elevated both in synovial fluid and serum [8]. Moreover, a relationship between baseline detectable IL-33 concentrations and the development of severe subclinical atherosclerosis in patients diagnosed with RA has been described [9].

Regarding genetic studies, polymorphisms located both in IL33 and IL1RL1 have been associated with autoimmunity. An association between IL33 rs3939286 and some immune-mediated diseases such as inflammatory bowel disease (IBD) has been described [10]. Additionally,
IL33 rs7025417 and IL33 rs4742170, which is in high linkage disequilibrium with IL33 rs7025417, have been related to coronary artery disease (CAD) and ischemic stroke in non-rheumatic Chinese individuals, respectively [11,12]. Also in the Chinese population, the IL33 rs7044343 polymorphism has been associated with RA [13]. Regarding IL1RL1, a relevant role of IL1RL1 rs2058660, IL1RL1 rs2310173 and IL1RL1 rs13015714 in the development of several inflammatory conditions such as IBD and ankylosing spondylitis has been proposed [10,14].

Taking into account all these considerations, in the present study we aimed to determine, for the first time, whether 6 genetic variants at IL33 and IL1RL1, previously associated with immune-mediated diseases, are involved in the risk of subclinical atherosclerosis in Spanish patients with RA.

Patients and Methods

2.1. Patients and Study Protocol

A set of 576 Spanish patients with RA were included in the present study. Blood samples were obtained from patients recruited from Hospital Lucus Augusti (Lugo) and Hospital Marqués de Valdecilla (Santander) in Northern Spain. A subject’s written consent was obtained according to the declaration of Helsinki, and the study was approved by the Ethics Committees of clinical research of Galicia (CAEI) for Hospital Lucus Augusti in Lugo and Cantabria (CEIC) for Hospital Universitario Marqués de Valdecilla in Santander.

All the patients fulfilled the 2010 classification criteria for RA [15]. In all the cases, patients were assessed for 3 polymorphisms within IL33 (rs3939286, rs7025417 and rs7044343) and 3 genetic variants located within IL1RL1 (rs2058660, rs2310173, rs13015714). Also, cIMT was determined by carotid ultrasound (US) technology. Information on the main demographic data, clinical characteristics, CV risk factors and CV events of the patients enrolled in the study is shown in Table 1. Definitions of CV events (ischemic heart disease, heart failure, cerebrovascular accident or peripheral arteriopathy) and those for traditional CV risk factors were established as previously described [2,6].

2.2. Genotyping

Genomic deoxyribonucleic acid (DNA) from all patients was extracted from peripheral white blood cells using standard procedures.

The selection of single-nucleotide polymorphisms (SNPs) was based on their position with regards to the IL33-IL1RL1 region and their previously reported associations with several inflammatory diseases. Following these criteria, we performed a tagging using data from HapMap project (http://www.hapmap.org) and Haplovie software version 4.2 and considering r² >0.8, haplotype frequency >5%, minor allele frequency >10%) (data in S1 and S2 figs). After that, we identified 6 polymorphisms, 3 in the IL33 region (rs3939286 (C__2762168_10), rs7025417 (C__31940410_20) and rs7044343 (C__29340326_10)) and 3 genetic variants located within IL1RL1 (rs2058660, rs2310173, rs13015714). Also, cIMT was determined by carotid ultrasound (US) technology. Information on the main demographic data, clinical characteristics, CV risk factors and CV events of the patients enrolled in the study is shown in Table 1. Definitions of CV events (ischemic heart disease, heart failure, cerebrovascular accident or peripheral arteriopathy) and those for traditional CV risk factors were established as previously described [2,6].

All genetic variants were genotyped with TaqMan predesigned single nucleotide polymorphism genotyping assays in a 7900 HT Real-Time polymerase chain reaction (PCR) system,
according to the conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA).

Negative controls and duplicate samples were included to check the accuracy of genotyping.

### 2.3. Carotid US Examination

The measurement of the cIMT was performed by carotid US. Patients from Santander were assessed using a commercially available scanner, Mylab 70, Esaote (Genoa, Italy) equipped with 7–12 MHz linear transducer and the automated software guided technique radiofrequency—Quality Intima Media Thickness in real-time (QIMT, Esaote, Maastricht, Holland)—[16]. Patients from Lugo were assessed using high-resolution B-mode ultrasound, Hewlett Packard SONOS 5500, with a 10-MHz linear transducer as previously reported [17]. cIMT was measured at the far wall of the right and left common carotid arteries, 10 mm from the carotid bifurcation, over the proximal 15 mm-long segment. cIMT was determined as the average of three measurements in each common carotid artery. The final cIMT was the largest average cIMT (left or right). Agreement between the two US methods in patients with RA was
Previously reported [18]. Experts with high experience and close collaboration in the assessment of subclinical atherosclerosis in RA from Lugo and Santander performed the studies.

2.4. Statistical Analysis

All genotype data were checked for deviation from Hardy-Weinberg equilibrium (HWE) using http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. cIMT values were displayed as mean ± standard deviation (SD). The association between genotypes and alleles frequencies of the IL33 rs3939286, IL33 rs7025417, IL33 rs7044343, IL1RL1 rs2058660, IL1RL1 rs2310173 and IL1RL1 rs13015714 polymorphisms and cIMT values was tested using unpaired t test to compare between 2 groups and one-way analysis of variance (ANOVA) to compare among more than two groups. Comparisons of means of cIMT values were adjusted for sex, age at the time of US study, follow-up time and center as potential confounders using analysis of covariance (ANCOVA). Statistical significance was defined as \( p < 0.05 \). All analyses were performed with STATA statistical software 12/SE (Stata Corp., College Station, TX, USA).

Results

All genotype distributions were in Hardy-Weinberg equilibrium. The genotyping success was greater than 97% in all the cases.

Results of the comparisons between IL33 rs3939286, IL33 rs7025417, IL33 rs7044343, IL1RL1 rs2058660, IL1RL1 rs2310173 and IL1RL1 rs13015714 according to cIMT values are shown in Table 2. RA patients carrying the TT genotype of the IL33 rs3939286 polymorphism had lower cIMT values than those homozygous for the CC genotype (mean ± SD: 0.71 ± 0.14 mm versus 0.76 ± 0.16 mm, respectively) whereas patients carrying the CT genotype had intermediate cIMT values (mean ± SD: 0.73 ± 0.17 mm) (Table 2). Moreover, RA patients carrying the mutant allele T of the IL33 rs3939286 polymorphism exhibited significantly lower cIMT values than those carrying the wild allele C (mean ± SD: 0.72 ± 0.16 mm versus 0.75 ± 0.18 mm respectively; \( p = 0.04 \)) (Table 2).

Since sex, age at the time of US study, follow-up time and center may act as potential confounders of the results derived from the US assessment; adjustment for these potential confounders was performed by an ANCOVA model. Interestingly, even after adjustment for potential confounders, both genotype and allele frequencies of the IL33 rs3939286 polymorphism were statistically significant (\( p = 0.006 \) and \( p = 0.0023 \), respectively) evidencing that the potential effect conferred by IL33 rs3939286 polymorphism may be independent of confounder factors (Table 2). Nevertheless, no significant association between IL33 rs7025417, IL33 rs7044343, IL1RL1 rs2058660, IL1RL1 rs2310173 and IL1RL1 rs13015714 and cIMT values was observed (Table 2).

Discussion

CV disease is the most common cause of premature mortality in patients with RA being a consequence of accelerated atherosclerosis [1]. In the last years, several studies have been focused on the assessment of biologic mechanisms that influence the risk of CV disease in RA. Accordingly, several genetic markers associated with the risk of subclinical atherosclerosis and CV disease in RA have recently been identified [2,4–5].

Outside the HLA region, cytokine pathway genes, which have critical modulatory effects on innate and adaptive immunity, have been shown to represent a key component of the genetic network associated with immune-mediated processes.
IL-33-IL-1RL1 pathway is a key proinflammatory mediator that may play a pathogenic role in RA [8]. Moreover, detectable IL-33 plasma concentrations at the time of disease diagnosis were found to predict the presence of severe subclinical atherosclerosis in the extended follow-up of patients with RA [9]. Taking into account these considerations and the implication of 6 genetic IL33-IL1RL1 variants in the susceptibility to several inflammatory diseases [10–14], we aimed to determine the potential association of these genetic polymorphisms with subclinical atherosclerosis in RA. Interestingly, our results suggest a potential protection effect of the mutant IL33 rs3939286T allele in the risk of subclinical atherosclerosis, established by the assessment of cIMT, in patients with RA. This association remained statistically significant after adjustment for potential confounders, evidencing that this potential effect may be independent of sex, age at the time of the carotid US study, follow-up time and center. It is worth noting that according to the public database RegulomeDB the IL33 rs3939286 genetic variant appears to be a regulatory DNA element [19]. Consequently, it could be plausible to think that the mutant IL33 rs3939286T allele might influence the development of subclinical atherosclerosis by regulating the expression of IL33 and, at last, by decreasing IL-33 levels. Despite having a cohort that might be considered relatively small, our study encompassed the largest series of RA patients with cIMT data ever assessed for genetic studies.

In keeping with our findings, recent studies have described a protective effect of the mutant allele of another IL33 gene polymorphism, rs7025417, in the risk of CAD in a Chinese population and also in the development of giant cell arteritis in Europeans [11,20]. Since IL33 rs3939286 and IL33 rs7025417 genetic variants are located in different haplotype blocks and present a low linkage disequilibrium (LD) (D’ = 0.13 and r² = 0.01), we speculate that either they are independent susceptibility factors for the different phenotypes or they might be tagging the real causal variant.

### Table 2. Association between IL33-IL1RL1 polymorphisms and cIMT values in RA patients.

| IL33 SNP | Genotype/Allele | cIMT mm mean ± SD (n) | p     | P*     | IL1RL1 SNP | Genotype/Allele | cIMT mm mean ± SD (n) | p     | P*     |
|----------|-----------------|-----------------------|-------|--------|------------|-----------------|----------------------|-------|--------|
| rs3939286 | CC              | 0.76 ± 0.16 (258)     | 0.10  | 0.006  | rs2058660  | AA              | 0.74 ± 0.17 (325)    | 0.91  | 0.58   |
|          | CT              | 0.73 ± 0.17 (259)     |       |        |            | AG              | 0.73 ± 0.18 (213)    |       |        |
|          | TT              | 0.71 ± 0.14 (50)      |       |        |            | GG              | 0.75 ± 0.16 (36)     |       |        |
|          | C               | 0.75 ± 0.18 (775)     | 0.04  | 0.0023 | rs2310173  | A               | 0.74 ± 0.17 (863)    | 0.95  | 0.40   |
|          | T               | 0.72 ± 0.16 (359)     |       |        |            | G               | 0.74 ± 0.18 (285)    |       |        |
| rs7025417 | TT              | 0.75 ± 0.18 (372)     | 0.15  | 0.16   | rs13015714 | TT              | 0.74 ± 0.17 (154)    | 0.93  | 0.76   |
|          | TC              | 0.72 ± 0.17 (172)     |       |        |            | GT              | 0.74 ± 0.18 (280)    |       |        |
|          | CC              | 0.76 ± 0.20 (21)      |       |        |            | TT              | 0.74 ± 0.18 (142)    |       |        |
|          | T               | 0.74 ± 0.18 (916)     | 0.23  | 0.16   |            | G               | 0.74 ± 0.17 (588)    | 0.77  | 0.45   |
|          | C               | 0.73 ± 0.17 (214)     |       |        |            | T               | 0.74 ± 0.18 (564)    |       |        |
| rs7044343 | TT              | 0.73 ± 0.17 (280)     | 0.35  | 0.33   | rs13015714 | TT              | 0.74 ± 0.18 (322)    | 0.88  | 0.39   |
|          | TC              | 0.74 ± 0.17 (229)     |       |        |            | TG              | 0.73 ± 0.18 (215)    |       |        |
|          | CC              | 0.77 ± 0.20 (54)      |       |        |            | TT              | 0.74 ± 0.17 (37)     |       |        |
|          | T               | 0.73 ± 0.17 (789)     | 0.20  | 0.26   |            | T               | 0.74 ± 0.18 (859)    | 0.73  | 0.29   |
|          | C               | 0.75 ± 0.18 (337)     |       |        |            | G               | 0.73 ± 0.18 (289)    |       |        |

cIMT: carotid intima-media thickness; RA: Rheumatoid arthritis; SNP: Single nucleotide polymorphism; SD: Standard deviation. Significant results are highlighted on bold.

*p-value after adjusting for sex, age at the time of ultrasonography study, follow-up time and center.

doi:10.1371/journal.pone.0143153.t002
The results obtained in the present study provide additional evidence on the potential role that genetic factors may play in the development of CV disease in RA. The search for genetic markers associated with CV disease in RA may be important for a better characterization of RA patients at risk of CV disease. They may be useful to predict disease outcome at diagnosis of RA and to establish future therapeutic targets to decrease the risk of CV disease in RA patients.

In conclusion, our results may suggest a potential protective effect of the IL33 rs3939286 allele T in the risk of subclinical atherosclerosis in patients with RA.

Supporting Information

S1 Fig. Position of the 3 tag IL33 polymorphisms analyzed in our study. The diamond represents the linkage disequilibrium degree between polymorphisms. The color indicates the D' (a redder color represents a higher D'). Data was obtained from HapMap project (http://www.hapmap.org) and Haploview software version 4.2 and considering r² > 0.8, haplotype frequency > 5%, minor allele frequency > 10%.

S2 Fig. Position of the 3 tag IL1RL1 polymorphisms analyzed in our study. The diamond represents the linkage disequilibrium degree between polymorphisms. The color indicates the D' (a redder color represents a higher D'). Data was obtained from HapMap project (http://www.hapmap.org) and Haploview software version 4.2 and considering r² > 0.8, haplotype frequency > 5%, minor allele frequency > 10%.

Acknowledgments

We wish to thank all the patients with RA that participated to make this study possible. We want to specially thank Patricia Fuentevilla Rodríguez, Virginia Portilla González, M. Luisa López, M. Jesús Ibañez and Sara Olavarria for their technical assistance.

Author Contributions

Conceived and designed the experiments: FG RLM SRM MRV MGB BU JAMF VM TP RB JJAS MARH MDMS BTS IFA EV FDC SC AC CGJ JL JM MAGG. Performed the experiments: FG RLM SRM MRV MGB BU JAMF VM TP RB JJAS MARH MDMS BTS IFA EV FDC SC AC CGJ JL JM MAGG. Analyzed the data: FG RLM SRM MRV MGB BU JAMF VM TP RB JJAS MARH MDMS BTS IFA EV FDC SC AC CGJ JL JM MAGG. Contributed reagents/materials/analysis tools: FG RLM SRM MRV MGB BU JAMF VM TP RB JJAS MARH MDMS BTS IFA EV FDC SC AC CGJ JL JM MAGG. Wrote the paper: FG RLM SRM MRV MGB BU JAMF VM TP RB JJAS MARH MDMS BTS IFA EV FDC SC AC CGJ JL JM MAGG.

References

1. Gonzalez-Gay MA, Gonzalez-Juanatey C, Martin J (2005) Rheumatoid arthritis: a disease associated with accelerated atherogenesis. Semin Arthritis Rheum 35: 8–17. PMID: 16084219
2. Gonzalez-Gay MA, Gonzalez-Juanatey C, Lopez-Diaz MJ, Píñeiro A, Garcia-Porrua C, Miranda-Filloy JA, et al. (2007) HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis. Arthritis Rheum 57: 125–32. PMID: 17266100
3. Dessein PH, Norton GR, Woodiwiss AJ, Joffe BI, Wolfe F (2007) Influence of nonclassical cardiovascular risk factors on the accuracy of predicting subclinical atherosclerosis in rheumatoid arthritis. J Rheumatol 34: 943–51. PMID: 17444592
4. López-Mejías R, Genre F, García-Bermúdez M, Corrales A, González-Juanatey C, Llorca J, et al. (2013) The ZC3HC1 rs11556924 polymorphism is associated with increased carotid intima-media thickness in patients with rheumatoid arthritis. Arthritis Res Ther 15: R152. doi: 10.1186/ar4335 PMID: 24286297

5. López-Mejías R, García-Bermúdez M, González-Juanatey C, Castañeda S, Miranda-Filloy JA, Gómez-Vaquero C, et al. (2012) NFKB1-94ATTG ins/del polymorphism (rs28362491) is associated with cardiovascular disease in patients with rheumatoid arthritis. Atherosclerosis 224: 426–9. doi: 10.1016/j.atherosclerosis.2012.06.008 PMID: 22742859

6. González-Juanatey C, Llorca J, Martín J, González-Gay MA (2009) Cardiot intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis. Semin Arthritis Rheum 38: 366–71. doi: 10.1016/j.semarthrit.2008.01.012 PMID: 18336869

7. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. (2012) Disease-associated functions of IL-33: the new kid in the IL-1 family. Immunity 23: 479–90.

8. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, et al. (2008) IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proc Natl Acad Sci U S A 105: 10913–8. doi: 10.1073/pnas.0801898105 PMID: 18667700

9. Shen J, Shang Q, Wong CK, Li EK, Wang S, Li RJ, et al. (2015) IL-33 and soluble ST2 levels as novel predictors for remission and progression of carotid plaque in early rheumatoid arthritis: A prospective study. Semin Arthritis Rheum 45: 18–27. doi: 10.1016/j.semarthrit.2015.02.001 PMID: 25798875

10. Latiano A, Palmieri O, Pastorelli L, Vecchi M, Pizarro TT, Bossa F, et al. (2013) Associations between genetic polymorphisms in IL-33, IL1R1 and risk for inflammatory bowel disease. PloS one 8: e62144. doi: 10.1371/journal.pone.0062144 PMID: 23634226

11. Tu X, Nie S, Liao Y, Zhang H, Fan Q, Xu C, et al. (2013) The IL-33-IL1RL1L pathway is associated with coronary artery disease in a Chinese Han population. Am J Hum Genet 93: 652–60. doi: 10.1016/j.ajhg.2013.08.009 PMID: 24075188

12. Guo L, Zhou X, Guo X, Zhang X, Sun Y (2013) Association of interleukin-33 gene single nucleotide polymorphisms with ischemic stroke in north Chinese population. BMC Med Genet 14: 109. doi: 10.1186/1471-2350-14-109 PMID: 24107076

13. Li C, Mu R, Guo J, Wu X, Tu X, Liu X, et al. (2014) Genetic variant in IL33 is associated with susceptibility to rheumatoid arthritis. Arthritis Res Ther 16: R105. doi: 10.1186/ar4554 PMID: 24779919

14. Reveille JD, Sims AM, Danoy P, Evans DM, Leo P, Pointon JJ, et al. (2010) Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 42: 123–7. doi: 10.1038/ng.1513 PMID: 20682062

15. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. (2010) 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 69: 1580–8. doi: 10.1136/ard.2010.138461 PMID: 20699241

16. Corrales A, Parra JA, González-Juanatey C, Rueda-Gotor J, Blanco R, Llorca J, et al. (2013) Cardiovascular risk stratification in rheumatic diseases: carotid ultrasound is more sensitive than Coronary Artery Calcification Score to detect subclinical atherosclerosis in patients with rheumatoid arthritis. Ann Rheum Dis 72: 1764–70. doi: 10.1136/annrheumdis-2013-203668 PMID: 23852762

17. González-Juanatey C, Llorca J, García-Porrua C, Martin J, Gonzalez-Gay MA (2006) Effect of anti-tumor necrosis factor alpha therapy on the progression of subclinical atherosclerosis in severe rheumatoid arthritis. Arthritis Rheum 55: 150–3. PMID: 16463428

18. Naredo E, Móller I, Gutiérrez M, Bong DA, Cobo T, Corominas H, et al. (2011) Multi-examiner reliability of automated radio frequency-based ultrasound measurements of common carotid intima-media thickness in rheumatoid arthritis. Rheumatology (Oxford) 50: 1860–4.

19. Boyle AP, Hong EL, Hariharavan M, Cheng Y, Schaub MA, Kasowski M, et al. (2012) Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 22: 1790–7. doi: 10.1101/gr.137323.112 PMID: 22955989

20. Márquez A, Solans R, Hernández-Rodríguez J, Cid MC, Castañeda S, Ramentol M, et al. (2014) A candidate gene approach identifies an IL33 genetic variant as a novel genetic risk factor for GCA. PLoS One 9: e113476. doi: 10.1371/journal.pone.0113476 PMID: 2409453