Interferon regulatory factor 5 gene variants rs2004640 and rs4728142 are associated with carotid intima media thickness but not with cardiovascular events in rheumatoid arthritis

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Abstract

Objective

Rheumatoid arthritis (RA) is associated with cardiovascular (CV) morbidity and mortality. Interferon regulatory factor 5 (IRF5) gene polymorphisms rs2004640 and rs4728142 have been associated with autoimmune diseases, but also with atherosclerosis. Differences in IRF5 gene expression can lead to the production of different interferons and might play a role in the atherogenic process in RA.

Methods

We investigated the effects of IRF5 gene variants rs2004640 and rs4728142 on clinical parameters related to atherosclerosis, such as cIMT (in subgroup n=101), and new CV events (in whole cohort n=353).

Results

For rs2004640, cIMT values at baseline were highest within the group of patients carrying the GG-genotype, followed by GT- and TT- genotypes, which was statistically significant. Over time patients with the TT-genotype had the highest increase in cIMT. For rs4728142 cIMT values were also the highest for patients with the GG-genotype at baseline, but the difference between the groups was not statistically significant. Over time the highest increase in cIMT was in the patients with the AA-genotype. Both rs2004640 and rs4728142 were not associated with new CV events during follow-up.

Conclusion

IRF5 alleles are associated with changes in cIMT, but not with new CV events in RA. Although these findings implicate a role of the IRF5 transcription pathway in atherosclerosis, IRF5 single nucleotide polymorphisms do not appear to increase the risk of future CV events.

Key words

interferon regulatory factor 5, single nucleotide polymorphisms, rheumatoid arthritis, cardiovascular disease

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Introduction

Rheumatoid arthritis (RA) is associated with increased cardiovascular (CV) morbidity and mortality (1-3), in part due to the presence of traditional CV risk factors (4), but also due to RA-related inflammation that is assumed to accelerate atherosclerosis. However, the exact mechanisms behind this phenomenon remains unknown (5, 6). Hence, the search for pathways linking RA to CV disease (CVD) is relevant. Interferon regulatory factor 5 (IRF5) gene polymorphisms, also known as single nucleotide polymorphisms (SNPs), rs2004640 and rs4728142 have been associated with autoimmune diseases, such as systemic lupus erythematosus (SLE) (7-10), RA (11-13) and multiple sclerosis (MS) (14), but also with atherosclerosis (15, 16). *IRF5* is a member of a family of transcription factors that controls inflammatory and immune responses through activation of toll like receptors (TLRs) (17). Furthermore, *IRF5* gene expression is involved in the type I interferon (IFN) pathway, leading to the production of different interferons (IFN) involved in the production of proinflammatory cytokines (17). Interferons (IFNs) are a group of cytokines that can both inhibit and promote vascular smooth cell proliferation, depending on the type of IFN pathway that is activated (18-22). Both IRF5 rs2004640 and rs4728142 SNPs can affect *IRF5* gene expression by alternative splicing of the *IRF5* gene, causing the production of different IFN types and therefore possibly playing a role in the atherogenic process in RA (7, 8). In this study, we investigated the effects of IRF5 gene variants on clinical parameters related to atherosclerosis (*i.e.* carotid intima media thickness (cIMT)) and new CV events in RA patients.

Patients and methods

Study population

The CARRE study is a prospective cohort study investigating CVD and its risk factors in RA patients (23). In 2000, a random sample of 353 RA patients registered at Reade (former Jan van Breemen Institute) in Amsterdam, the Netherlands, was drawn. Patients fulfilled the 1987 American College of Rheumatology classification criteria for RA, and were aged between 50 and 75 (23, 24). An ultrasound study of the carotid artery was performed in 2001 in a randomly selected subgroup of 101 patients. The local ethics committee and institutional review boards of the VU University Medical Center and Slotervaart Hospital/Reade in Amsterdam, the Netherlands, approved the study protocol and all participants gave their written informed consent to participate.

DNA extraction and genotyping

Total DNA was extracted from EDTA blood from 353 RA patients using Qiagen's DNAeasy blood and tissue kit (Qiagen) according to the manufacturer instructions. The IRF5 gene variants rs2004640 and rs4728142 were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, CA) according to the manufacturers protocol. Allelic discrimination was performed using an ABI Prism 7900HT sequence Detection system.

Assessment of CV risk factors and RA related factors

CVD history, medical history and medication use was obtained as previously described by Peters *et al.* (25). Total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), triglycerides (TG), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and IgM rheumatoid factor (IgM-RF) antibodies were determined as describes previously (26). TC/HDLc ratio was calculated by dividing TC with HDLc. Body mass index (BMI) was calculated as the ratio of weight and squared height. Hypertension was defined as a systolic blood pressure (SBP) over 140 mmHg and/or a diastolic blood pressure (DBP) over 90 mmHg and/or the current use of antihypertensive medication. Physical examination was performed to determine the Disease Activity Score in 28 joints (DAS28) (27). Radiographs of hands and feet were obtained to investigate the presence of erosions.

Carotid intima media thickness measurement

cIMT was assessed with Artlab echo

tracking system using a 7.5-MHz linear probe, connected to a computer equipped with vessel wall movement detection software and an acquisition system (Esaote Europe BV). After localisation of the common carotid artery, cross-sectional measurements were performed 10mm proximal of the carotid bifurcation as described previously (28).

Assessment of new CV events

As described previously, new CV events were registered according to the International Statistical Classification of Diseases and Related Health Problems 9th revision (ICD-9 codes, 410.0-410.9, 435.9, 436, 443.9 and 798) (29). CV events were verified in medical records and included coronary heart disease (*i.e.* myocardial infarction, percutaneous coronary intervention, coronary angiography with significant stenosis, stent placement or coronary artery bypass graft), cerebral arterial disease (*e.g.* cerebrovascular accident, transient ischaemic attack or carotid endarterectomy) or peripheral arterial disease (*e.g.* ankle brachial pressure index <0.50, peripheral arterial reconstructive surgery or limb amputation) (29). Patients were censored after the first CV event or death due to other reasons. The patients who were lost to follow-up were censored at the date of their last follow-up visit. The remainder of the patients was censored at study cessation time on March 1, 2015 (29).

Statistical analysis

Patients were grouped according to their SNP rs2004640 or rs4728142 allele distribution. Differences in demographics, CV- and RA-related factors between allele groups and genotypes were analysed using Students t-test, Chi-square test, Mann-Whitney U-test, and ANOVA as appropriate. Logistic regression analyses were used to investigate the association between IRF5 genotypes and cIMT in the subgroup of 101 patients and Cox proportional hazard models were used to investigate the association with new CVD events in the whole cohort of 353 patients. Longitudinal cIMT data over 3 time points (*i.e.* baseline, 3 year and 10 year measurements) was analysed using generalised estimation equations (GEE). The minor allele was used as the reference group in all analyses. The analyses were adjusted for demographic, CV- and RA-related factors on the basis of the literature (29) and differences between the groups identified at baseline. Statistical analyses were performed with IBM SPSS v. 23.0. *p*-values of <0.05 (two tailed) were considered statistically significant.

Table I. Baseline characteristics.

	cIMT cohort (n=101)	CARRE cohort (n=353)	*p*-value
Demographics			
Age, years	62 ± 7	63 ± 8	0.62
Female, no. (%)	61 (60.4)	232 (65.7)	0.32
Caucasian, no. (%)	97 (96)	332 (94.1)	0.69
RA characteristics			
RA duration, years	8 (5 – 11)	7 (4 – 10)	0.71
DAS28	3.5 ± 1.2	3.9 ± 1.4	0.31
ESR, mm/hr	12 (8 – 27)	18 (9 – 31)	0.03
CRP, mg/l	6 (3 – 15)	7 (3 – 18)	0.22
IgM-RF positive, no. (%)	72 (71.3)	256 (72.5)	0.81
ACPA positive, no (%)	58 (57.4)	187 (53)	0.56
Erosive disease, no. (%)	82 (81.2)	288 (81.6)	0.99
Medication use, no (%)			
Antihypertensive	25 (24.8)	94 (26.6)	0.69
Statin	11 (10.9)	40 (11.3)	0.90
Salicylic acid	18 (17.8)	56 (15.9)	0.64
Prednisone	13 (12.9)	58 (16.4)	0.49
cDMARDs	88 (87.1)	303 (85.8)	0.74
bDMARDs	11 (10.9)	34 (9.6)	0.71
CV parameters			
TC, mmol/L	5.7 ± 1.0	5.8 ± 1.1	0.55
HDLc, mmol/L	1.5 ± 0.5	1.5 ± 0.5	0.53
LDLc, mmol/L	3.6 ± 1.1	3.7 ± 1.0	0.54
TG, mmol/L	1.3 (1.0 – 1.7)	1.5 (1 – 1.8)	0.58
BMI, kg/m^2	26 ± 4	27 ± 5	0.28
Pack years	21 (3 – 39)	19 (2 – 36)	<0.01
Systolic BP, mmHg	142 ± 18	142 ± 20	0.96
Diastolic BP, mmHg	82 ± 7	81 ± 9	0.28
Diabetes, no. (%)	4 (4)	17 (4.8)	0.71
IMT, mm	0.812 ± 0.131	-NA	
Previous CVD, no. (%)	15 (14.9)	51 (14.4)	0.91
IRF5 minor allele frequencies			
Rs2004640 (G)	0.451	0.483	NA
Rs4728142 (A)	0.415	0.418	NA

DAS28: Disease Activity Score in 28 joints; cIMT: carotid intima media thickness; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; IgM-RF: IgM-rheumatoid factor; ACPA: anti-citrullinated protein antibody; cDMARDs: conventional disease-modifying anti-rheumatic drugs; bDMARDs: biologic disease-modifying anti-rheumatic drugs; TC: total cholesterol; HDLc: high-density lipoprotein cholesterol; LDLc: low-density lipoprotein cholesterol; TG: triglycerides; BMI: Body Mass Index; BP: blood pressure, CVD: cardiovascular disease; NA: not applicable.

Results

Patient characteristics

The 353 RA patients from the CARRE cohort were genotyped for IRF5 SNPs rs2004640 and rs4728142. Of these 353 patients, cIMT was determined in 101 patients, which is referred to as the 'cIMT cohort'. Genotyping failed in eight patients of the CARRE cohort, of which one patient was in de cIMT cohort. Both SNPs were in Hardy-Weinberg equilibrium. The baseline characteristics and allele frequencies of IRF5 rs2004640 and rs4728142 are shown in Table I.

Association of the rs2004640 genotype with RA- and CVD-related factors, cIMT and new CV events

First, we analysed whether rs2004640 was associated with CV risk factors. No significant associations with HDLc, LDLc, SBP, DBP, TC, HDLc, LDLc, TG, BMI, smoking or prevalent CV disease were observed (data not shown). Secondly, we tested whether IRF5 gene variants were associated with RA-related clinical parameters (*i.e.* RA duration,

DAS28, CRP, presence of erosions, presence of RF and/or ACPA). A higher percentage of patients homozygous for the rs2004640 G-allele were positive for RF compared to patients with a GT- or TT-genotype (respectively 82%, 67% and 70%, p=0.048). No significant associations were observed between IRF5 genotypes, and other RA-related characteristics (data not shown). For rs2004640, the highest cIMT values at baseline were observed in patients with the GG-genotype, followed by the GT- and TT- genotypes (0.874±0.142, 0.806±0.125, 0.785±0.126). The difference in cIMT between patients carrying the GG- *vs*. TT-genotype was significant (p=0.03, Fig. 1).

Patients with the TT-genotype were more likely to experience an increase in cIMT (median yearly progression in millimetres 0.02 (0.02–0.05)) over time when compared to the GG-genotype (median yearly progression 0.003 (-0.03–0.07) and respectively OR 2.84, 95%CI 1.07–7.50, p=0.035 and OR 0.26, 95%CI 0.08–0.75, p=0.013). This was also significant after correction for CV risk factors (age, sex, SBP, TC/HDLc-ratio, current smoking and aspirin use) with TT-genotype OR 3.03, 95%CI 1.06–8.65, p=0.038 and GG-genotype OR 0.22, 95%CI 0.07–0.69, p=0.01.

99 of the 353 patients developed a new CV event over a median follow-up duration of 9 (5–11) years. In the crude Cox regression analyses rs2004640 genotypes were not associated with new CV events (reference GG-genotype, GT-genotype p=0.59 and TT-genotype p=0.76).

Association of the rs4728142 genotype with RA- and CVD-related factors, cIMT and new CV events

For rs4728142 there were no significant associations with CV risk factors HDLc, LDLc, SBP, DBP, TC, HDLc, LDLc, TG, BMI, smoking or prevalent CV disease were observed (data not shown). There were no associations between RA-related risk factors and rs4728142 (data not shown). No significant differences were observed between cIMT values for the genotypes of rs4728142 (AA 0.775±0.137, AG 0.805±0.129, GG 0.843±0.131, p=0.22, Fig. 1). For rs4728142, in patients with the GG-genotype there was a trend for less increase of cIMT (median yearly progression 0.01 (-0.002–0.05) *vs*. 0.003 (-0.02–0.06)) over time (OR 0.43, 95%CI 0.18–1.02, p=0.057). After correction for traditional risk factors (as described above) there was a significant lower increase in cIMT for the GG-genotype with an OR of 0.34, 85%CI 0.14–0.88, p=0.025. For the other genotypes there was no significant increase or decrease in cIMT (AA OR 2.02, 95%CI 0.59–6.96, p=0.26, AG OR 1.51, 95%CI 0.68 – 3.37, p=0.31; after adjustment CV risk factors respectively ORs of 2.85, 95%CI 0.72–11.2, p=0.13 and 1.66, 95%CI 0.69–3.99, p=0.25). The rs4728142 genotypes were not associated with the development of new CV events over time in the crude Cox regression analyses (reference AA-genotype, AG-genotype p=0.51, GG-genotype p=0.90).

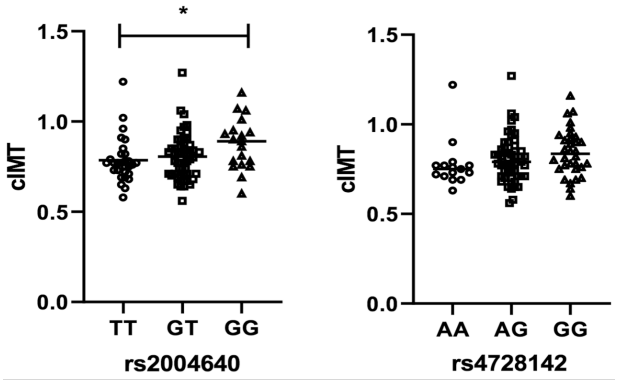

Fig. 1. cIMT values per genotype for rs2004640 and rs4728142.*p<0.05

Discussion

In our current study, both IRF5 rs2004640 and rs4728142 GG-genotypes were associated with cIMT and patients with the GG-genotype had the highest cIMT values at baseline, but the greatest increase in cIMT over time was seen in the TT- and AA-genotypes. However, these genotypes were not associated with the development of new CV events during follow-up. One of the explanations for this observation could be the sample size of our study population, which could be too small to detect an effect of these SNPs on CVD risk. Another explanation could be that IRF5 SNPs might not influence CVD risk directly or that their effect on CVD risk is very small. For rs2004640, a previous study described fewer CV events in patients with the GG-genotype (30). In line with this, Malarstig *et al*. identified *IRF5* mRNA expression in human carotid plaques, including the expression of SNPs rs2004640 and rs4728142, but they were not associated with a risk of (unstable) coronary artery disease or recurrent CV events in patients who had presented with unstable coronary artery disease (15). In our study, we have also identified an association between IRF5 SNPs and surrogate markers of atherosclerotic disease (*i.e*. cIMT), but not with new CVD over a median follow-up period of 9 years. This is an interesting finding, as IRF5 gene variants are detectible in atherosclerotic plaques and may have different effects on the atherosclerotic process, but they do not seem to be associated with an increased event risk. *IRF5* is a master regulator of type I IFN activity and functions as a transcription factor when phosphorylated, leading to expression of downstream interferon response genes, including the production of type I IFN itself and cytkonines such as interleukin-6 (IL-6), tumour necrosis factor (TNF), interleukin-12 (IL-12) and interleukin-23 (IL-23). The splicing of *IRF5* is highly complex and multiple *IRF5* isoforms are initiated at each promoter. Different isoforms can contain either exon 1a, 1b or 1c, depending on the promoter where transcription is initiated. Furthermore, it is known that the *IRF5* isoforms differ in their ability to transactivate type I IFN genes, *i.e*. IFNα or IFNβ (31). IFNβ and IFNα, have been described to have both anti- and proatherosclerotic effects in several *in vitro* studies (21, 22, 32). The rs2004640 IFR5 SNP is locat-

ed 2bp near the intron-exon boundary for exon 1b and creates an exon donor splice site, which enables transcription of exon 1b. Specifically the T-allele enables transcription of exon 1b, which is associated with higher mRNA levels of *IRF5* (7). Thus, the IRF5 rs2004640 T-allele is likely to enhance the expression of *IRF5* and successively type I IFNs. In our study, we found a greater increase in cIMT in patient with the TT-allele, but this was not associated with new CV events during follow-up. rs4728142 is located on the promotor region of *IRF5*, and has previously been associated with autoimmune disease such as SLE and MS (7, 14). The exact function of IFNs in the development of CVD remains to be elucidated.

Some limitations need to be considered. Our study had a small sample size which could have influenced our results as mentioned above, specifically the power to detect significant differenced between the different IRF5 SNP genotypes. This could be one of the reasons for finding an association between cIMT and both IRF5 SNPs, but not with future CVD.

To our knowledge, this is the first study reporting an association between IRF5 alleles and cIMT, but not with new CV events in a cohort of RA patients followed for a longer period of time. Although, these findings seem to implicate a role of *IRF5* genetics in the development of artherosclerosis, *IRF5* gene variants do not appear to increase the risk of future CVD events.

References

1. BACON PA, STEVENS RJ, CARRUTHERS DM, YOUNG SP, KITAS GD: Accelerated atherogenesis in autoimmune rheumatic diseases. *Autoimmun Rev* 2002; 1: 338-47.
2. PETERS MJ, VAN HALM VP, VOSKUYL AE *et al*.: Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. *Arthritis Rheum* 2009; 61: 1571-9.
3. VAN DOORNUM S, MCCOLL G, WICKS IP: Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis? *Arthritis Rheum* 2002; 46: 862-73.
4. DEL RINCON ID, WILLIAMS K, STERN MP, FREEMAN GL, ESCALANTE A: High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 2001; 44 2737-45.
5. GONZALEZ-GAY MA, GONZALEZ-JUANATEY C, MARTIN J: Rheumatoid arthritis: a disease associated with accelerated atherogenesis. *Semin Arthritis Rheum* 2005; 35: 8-17.
6. SATTAR N, MCCAREY DW, CAPELL H, MCINNES IB: Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation* 2003; 108: 2957-63.
7. GRAHAM RR, KOZYREV SV, BAECHLER EC *et al*.: A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. *Nat Genet* 2006; 38: 550-5.
8. GRAHAM RR, KYOGOKU C, SIGURDSSON S *et al*.: Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. *Proc Natl Acad Sci USA* 2007; 104: 6758-63.
9. SIGURDSSON S, GORING HH, KRISTJANSDOTTIR G *et al*.: Comprehensive evaluation of the genetic variants of interferon regulatory factor 5 (IRF5) reveals a novel 5 bp length polymorphism as strong risk factor for systemic lupus erythematosus. *Hum Mol Genet* 2008; 17: 872-81.
10. SIGURDSSON S, NORDMARK G, GORING HH *et al*.: Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *Am J Hum Genet* 2005; 76: 528-37.
11. DIEGUEZ-GONZALEZ R, CALAZA M, PEREZ-PAMPIN E *et al*.: Association of interferon regulatory factor 5 haplotypes, similar to that found in systemic lupus erythematosus, in a large subgroup of patients with rheumatoid arthritis. *Arthritis Rheum* 2008; 58: 1264-74.
12. BO M, ERRE GL, NIEGOWSKA M *et al*.: Interferon regulatory factor 5 is a potential target of autoimmune response triggered by Epstein-barr virus and Mycobacterium avium subsp. paratuberculosis in rheumatoid arthritis: investigating a mechanism of molecular mimicry. *Clin Exp Rheumatol* 2018; 36: 376-81.
13. LOPEZ-MEJIAS R, CASTANEDA S, GONZALEZ-JUANATEY C *et al*.: Cardiovascular risk assessment in patients with rheumatoid arthritis: The relevance of clinical, genetic and serological markers. *Autoimmun Rev* 2016; 15: 1013-30.
14. KRISTJANSDOTTIR G, SANDLING JK, BONETTI A *et al*.: Interferon regulatory factor 5 (IRF5) gene variants are associated with multiple sclerosis in three distinct populations. *J Med Genet* 2008; 45: 362-9.
15. MALARSTIG A, SIGURDSSON S, ERIKSSON P *et al*.: Variants of the interferon regulatory factor 5 gene regulate expression of IRF5 mRNA in atherosclerotic tissue but are not associated with myocardial infarction. *Arterioscler Thromb Vasc Biol* 2008; 28: 975-82.
16. LOPEZ-MEJIAS R, CARMONA FD, GENRE F *et al*.: Identification of a 3'-untranslated genetic variant of RARB associated with carotid intima-media thickness in rheumatoid arthritis: a genome-wide association study. *Arthritis Rheumatol* 2019; 71: 351-60.
17. HONDA K, YANAI H, TAKAOKA A, TANIGUCHI T: Regulation of the type I IFN induction: a current view. *Int Immunol* 2005; 17: 1367-78.
18. MINTZER RJ, CROZE E, RUBANYI GM, JOHNS A: Differential effects of IFN-beta1b on the proliferation of human vascular smooth muscle and endothelial cells. *J Interferon Cytokine Res* 1998; 18: 939-45.
19. PALMER H, LIBBY P: Interferon-beta. A potential autocrine regulator of human vascular smooth muscle cell growth. *Lab Invest* 1992; 66: 715-21.
20. STEPHAN D, SAN H, YANG ZY *et al*.: Inhibition of vascular smooth muscle cell proliferation and intimal hyperplasia by gene transfer of beta-interferon. *Mol Med* 1997; 3: 593-9.
21. WESSELY R: Interference by interferons: Janus faces in vascular proliferative diseases. *Cardiovasc Res* 2005; 66: 433-43.
22. ZHANG LN, VELICHKO S, VINCELETTE J *et al*.: Interferon-beta attenuates angiotensin II-accelerated atherosclerosis and vascular remodeling in apolipoprotein E deficient mice. *Atherosclerosis* 2008; 197: 204-11.
23. PETERS MJ, VAN HALM VP, NURMOHAMED MT *et al*.: Relations between autoantibodies against oxidized low-density lipoprotein, inflammation, subclinical atherosclerosis, and cardiovascular disease in rheumatoid arthritis. *J Rheumatol* 2008; 35: 1495-9.
24. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
25. VAN HALM VP, PETERS MJ, VOSKUYL AE *et al*.: Rheumatoid arthritis versus diabetes as a risk factor for cardiovascular disease: a cross-sectional study, the CARRE Investigation. *Ann Rheum Dis* 2009; 68: 1395-400.
26. NIELEN MM, VAN SCHAARDENBURG D, REESINK HW *et al*.: Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004; 50: 380-6.
27. PREVOO ML, VAN 'T HOF MA, KUPER HH, VAN LEEUWEN MA, VAN DE PUTTE LB, VAN RIEL PL: Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-8.
28. PETERS MJ, VAN EIJK IC, SMULDERS YM *et al*.: Signs of accelerated preclinical atherosclerosis in patients with ankylosing spondylitis. *J Rheumatol* 2010; 37: 161-6.
29. AGCA R, HOPMAN L, LAAN KJC *et al*.: Cardiovascular event risk in rheumatoid arthritis compared with type 2 diabetes: a 15-year longitudinal study. *J Rheumatol* 2020; 47: 316-24.
30. GARCIA-BERMUDEZ M, LOPEZ-MEJIAS R, GENRE F *et al*.: Interferon regulatory factor 5 genetic variants are associated with cardiovascular disease in patients with rheumatoid arthritis. *Arthritis Res Ther* 2014; 16: R146.
31. MANCL ME, HU G, SANGSTER-GUITY N *et al*.: Two discrete promoters regulate the alternatively spliced human interferon regulatory factor-5 isoforms. Multiple isoforms with distinct cell type-specific expression, localization, regulation, and functi*on*. *J Biol Chem* 2005; 280: 21078-90.
32. GOOSSENS P, GIJBELS MJ, ZERNECKE A *et al*.: Myeloid type I interferon signaling promotes atherosclerosis by stimulating macrophage recruitment to lesions. *Cell Metab* 2010; 12: 142-53.