
Universiteit
Leiden
The Netherlands

The course of clinically suspect arthralgia and early rheumatoid arthritis : clinical features, imaging and genetics

Steenbergen, H.W. van

Citation
Steenbergen, H. W. van. (2016, November 8). *The course of clinically suspect arthralgia and early rheumatoid arthritis : clinical features, imaging and genetics*. Retrieved from https://hdl.handle.net/1887/44019

Version:	Not Applicable (or Unknown)
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/44019

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/44019 holds various files of this Leiden University dissertation.

Author: Steenbergen, H.W. van
Title: The course of clinically suspect arthralgia and early rheumatoid arthritis : clinical features, imaging and genetics
Issue Date: 2016-11-08

Does a genetic variant in *FOXO3A* predict a milder course of rheumatoid arthritis?

Hanna van Steenbergen, Solbritt Rantapää-Dahlqvist, Jessica van Nies, Ewa Berglin, Tom Huizinga, Peter Gregersen, Annette van der Helm-van Mil

9

Arthritis Rheumatol 2014;66:1678-81

The severity of rheumatoid arthritis (RA) is variable between patients, and the processes underlying these interindividual differences are scarcely understood. Although it has been observed that the severity of joint destruction is heritable [1], and several identified genetic risk factors have been replicated in independent cohorts [2,3], a large part of the total genetic effect is still unexplained. Unraveling the biologic processes that determine the course of RA increases our comprehension of disease progression and may convey novel targets for focused therapies.

Lee et al reported a milder disease course in patients carrying the *FOXO3A* minor allele (G) of rs12212067 [4]. That candidate gene study addressed genetic variants in the immune pathways of interleukin-2 (IL-2) and IL-7 and was initially performed in patients with Crohn's disease. It was observed that the rs12212067 minor allele was associated with higher transcription of FoxO3 in blood monocytes after lipopolysaccharide stimulation and with down-regulation of tumor necrosis factor α (TNFα) and other proinflammatory cytokines and up-regulation of IL-10. The relevance of FoxO3 in disease outcome was supported by associations with more severe malaria and less severe joint damage in patients with early RA[4].

FoxO3 is a transcription factor that is involved in the regulation of immune cell homeostasis [5]. Increased expression of FoxO3 in polymorphonuclear cells has been described in RA [6]. The results described by Lee et al are promising, because identification of predictors of interindividual differences in disease outcome is the Holy Grail of personalised medicine. This finding therefore requires replication in independent cohorts. The challenges within the field of RA severity are that large longitudinal cohorts with well-characterised data are scarce, and that cohorts of patients who were treated in an era when disease-modifying antirheumatic drugs (DMARDs) were less potent and strategies were not guided by the Disease Activity Score (DAS) are rare [7]. We examined rs12212067 in *FOXO3A* in relation to the severity of RA in multiple cohorts; the majority of patients studied were treated in the era before the introduction of biologic agents and DAS-guided therapy.

The main outcome of our study was radiographic progression. Five independent data sets were studied, comprising a total of 2,300 patients with RA and 5,512 radiographs. RA was defined according to the American College of Rheumatology 1987 revised criteria [8]. All patients gave informed consent, and approval was obtained from the local medical ethics committees.

The Leiden Early Arthritis Clinic (EAC) cohort comprised 597 patients with early RA, all of whom were included between 1993 and 2006 [9]. At baseline and at yearly follow-up visits over 7 years, a total of 3,143 sets of radiographs of the hands and feet were obtained. These radiographs were scored according to the Sharp-van der Heijde (SHS) method [10] by one reader in chronologic order (within-reader intraclass correlation coefficient [ICC] 0.91). The initial treatment strategy differed for different inclusion periods: patients included in 1993–1995 were initially treated with nonsteroidal anti-inflammatory drugs, patients included

in 1996–1998 were initially treated with hydroxychloroquine or sulfasalazine, and patients included in 1999–2006 were promptly treated with methotrexate [9].

The Umeå cohort consisted of 459 patients with early RA from Sweden, in whom RA was diagnosed between 1996 and 2010 [11]. A total of 868 sets of radiographs of the hands and feet obtained at baseline and year 2 were scored using the Larsen method [12], as described previously [11]. All patients were initially treated with methotrexate or sulfasalazine. Treatment with biologic agents during the 2-year follow-up period was uncommon (5.7%).

The North American Rheumatoid Arthritis Consortium (NARAC) study group comprised 384 unrelated patients in whom RA was diagnosed between 1953 and 2002 [13]. One set of radiographs of the hands was available for each patient. The radiographs were scored according to the SHS method (ICC 0.99).

The Wichita cohort consisted of 101 patients from a single practice in Wichita, Kansas, in whom RA was diagnosed between 1963 and 1999 [14]. Radiographic evaluations were not performed at protocolised time points; 358 sets of hand radiographs were obtained during the first 15 years after disease onset. These were scored in a known time order, using the SHS method (ICC 0.98).

The National Data Bank for Rheumatic Diseases (NDB) cohort comprised 759 patients from the US and Canada, in whom RA was diagnosed between 1944 and 1999 [15]. One set of radiographs of the hands was available for each patient. The radiographs were scored using the SHS method (ICC 0.98). The patients in the 3 North American cohorts were treated in an era when biologic agents were uncommon.

Genotyping in the Leiden EAC, Umeå, Wichita, and NDB cohorts was performed using the Illumina ImmunoChip according to the manufacturer's protocols, as previously described [16], and data for rs12212067 located on chromosome 6 were extracted. In the NARAC cohort, genotyping was performed using Illumina HapMap 500 BeadChips [13,17]. Data for rs12212067 were not available in the NARAC group, but data for a perfect proxy for this variant (rs11153120) were retrieved (r^2 1.00).

In all data sets, the radiographic scores were log-transformed before analyses to approximate a normal distribution. In each data set, the relative progression rate in patients with the rs12212067 minor allele was estimated, using patients with the common genotype as reference. An additive model was used. For the analyses in the cohorts with multiple measurements per patient (Leiden EAC, Umeå, and Wichita), a multivariate normal regression analysis was performed, with radiographic damage as the response variable [18]. For the data sets with one radiographic measurement per patient (NARAC and NDB), the estimated yearly progression rate (the total SHS divided by the number of disease-years at the time of radiography) was studied. Details on the statistical methodology and adjustment factors are available in the online Supplementary Methods (available on the Arthritis & Rheumatology website). Because all of the obtained effect sizes represented the relative

increase in the progression in joint destruction per year per minor allele, the effect sizes and standard errors of the individual analyses could be compared and combined in an inverse-variance weighted meta-analysis.

Because of the observation that the rs12212067 minor allele was associated with lower TNFα expression and higher production of anti-inflammatory interleukins [4], which presumably affect not only joint damage but also arthritis persistency, a second outcome measure, achieving sustained DMARD-free remission, was studied. Sustained DMARD-free remission is defined as the sustained absence of clinically detectable arthritis after discontinuation of DMARD therapy. This is described elsewhere and in the online Supplementary Methods. This stringent definition of remission is a proxy for cure [19]. Data on achieving DMARD-free remission were available only in the Leiden EAC cohort during 15 years of follow-up. Kaplan-Meier survival analyses were performed.

The characteristics of the patients are shown in the online Supplementary Table 1. In the largest cohort, consisting of 3,143 radiographs and 597 patients, rs12212067 was not statistically significantly associated with the severity of radiographic progression (p=0.14). No tendency toward less severe joint damage in the presence of the minor G allele was observed (Figure 1A). Significance was not obtained in any of the other cohorts. The meta-analysis did not reveal a significant association with radiographic progression (p=0.10), and the directionality of the effect sizes was not uniform (Figure 1B). When the secondary outcome of achieving sustained DMARD-free disease remission was evaluated, no significant association was observed (p=0.54) (Figure 1C).

Although the analyses described by Lee et al were performed in a mixed autoantibody-positive and antibody-negative population [4,20,21], we also performed analyses adjusted and stratified for the presence of anti–citrullinated protein antibodies (ACPA). In the analyses that were additionally adjusted for ACPA, rs12212067 was not significantly associated with the severity of radiographic progression (see online Supplementary Figure 1A). In the meta-analysis of the ACPA-positive subgroup, a significant result was obtained using the fixed-effects model but not the random-effects model; the directionality of the effect sizes was diverse (see online Supplementary Figure 1B). In the meta-analysis of the ACPA-negative subgroup, no association between rs12212067 and radiographic progression was observed. For the outcome of achieving sustained DMARD-free disease remission, no significance was observed in the ACPA-positive and ACPA-negative strata (p=0.77 and p=0.62, respectively).

In conclusion, from a clinical perspective, it is highly relevant to unravel the biology determining disease outcome. A recent study demonstrated a protective association between a genetic variant in *FOXO3A* and the severity of radiographic joint destruction in 2 early RA cohorts consisting of both ACPA-positive and ACPA-negative patients [4,20,21]. Using 5 independent RA cohorts, we could not replicate an association of *FOXO3A* with the severity of RA, implying that the initial observation in UK cohorts cannot be extrapolated to other

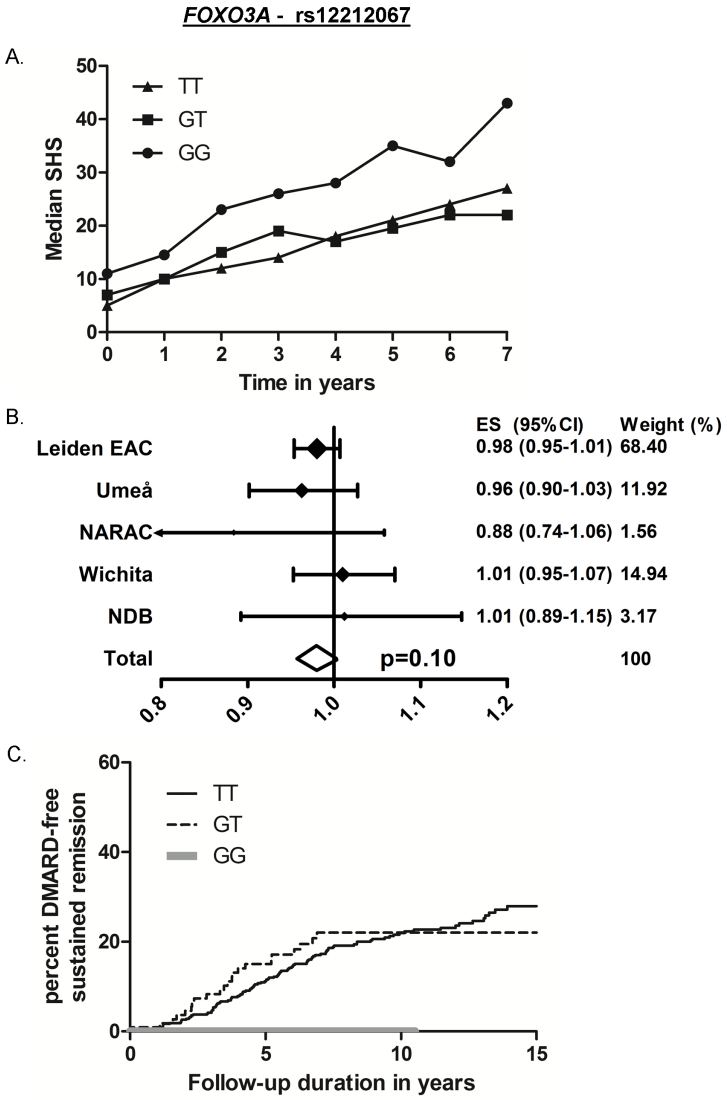

Figure 1. Relationship of *FOXO3A* rs12212067 genotypes with radiographic progression and DMARD-free sustained remission. (A) Median raw Sharp-van der Heijde (SHS) scores during 7 years of follow-up in patients in the Leiden EAC cohort. (B) Inverse-variance weighted meta-analysis of the annual radiographic progression rate in 5 cohorts, consisting of 2,300 patients and 5,512 radiographs (I^2 0.0%, p=0.59; for both the fixed-effects and random-effects models, p=0.10). (C) Frequency of achieving sustained DMARD-free remission in the Leiden EAC cohort (p=0.54 by log rank test). Only 6 patients had the GG genotype, and none of them achieved disease remission. The frequencies of the minor allele (G) were 9.3% in the Leiden EAC cohort, 12.0% in the Umeå cohort, 8.6% in the NARAC cohort, 9.3% in the Wichita cohort, and 10.5% in the NDB cohort. ES=effect size; 95% CI=95% confidence interval.

populations. This may reflect some differences in regulating progression in very early disease, which was the focus of the UK studies. Some data sets studied here were smaller than the UK cohorts; consequently, these individual cohorts were underpowered to replicate the signal individually. However, when the 5 data sets were combined, the number of radiographs was larger than that in the original study, and the meta-analysis was adequately powered to identify statistically significant differences and prevent false-negative results. The prevalence of ACPA in our cohorts was similar or higher than that in the original cohorts [20,21]. After stratification for ACPA, the p-value for a meta-analysis with a fixed-effects model was less than 0.05 within ACPA-positive patients; however, the directionality of the effect sizes was variable. The question of whether SNP rs12212067 in *FOXO3A* is associated with joint destruction in the ACPA-positive subgroup of patients with RA requires further investigation. The absence of an association of rs12212067 with sustained DMARD-free remission (the reverse of disease persistency) further supported the notion that *FOXO3A* is not a major factor regulating the severity of the course of RA.

SUPPLEMENTARY DATA

Supplementary data are published on the website of *Arthritis & Rheumatology*.

REFERENCES

1. Knevel R, Gröndal G, Huizinga TW, et al. Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study. Ann Rheum Dis 2012;71:707–9.
2. De Rooy DP, Zhernakova A, Tsonaka R, et al. A genetic variant in the region of MMP-9 is associated with serum levels and progression of joint damage in rheumatoid arthritis. Ann Rheum Dis 2014;73:1163–9.
3. Knevel R, de Rooy DP, Zhernakova A, et al. Association of Variants in IL2RA With Progression of Joint Destruction in Rheumatoid Arthritis. Arthritis Rheum 2013;65:1684–93.
4. Lee JC, Espéli M, Anderson CA, et al. Human SNP Links Differential Outcomes in Inflammatory and Infectious Disease to a FOXO3-Regulated Pathway. Cell 2013;155:57–69.
5. Hedrick SM. The cunning little vixen: Foxo and the cycle of life and death. Nat Immunol 2009;10:1057–63.
6. Turrel-Davin F, Tournadre A, Pachot A, et al. FoxO3a involved in neutrophil and T cell survival is overexpressed in rheumatoid blood and synovial tissue. Ann Rheum Dis 2010;69:755–60.
7. Van der Heijde DM, van 't Hof MA, van Riel PL, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 1990;49:916–20.
8. 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.
9. De Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? Rheumatology 2011;50:93–100.
10. Van der Heijde DM, van Riel PL, Nuver-Zwart IH, et al. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. Lancet 1989;1:1036–8.
11. Innala L, Kokkonen H, Eriksson C, et al. Antibodies Against Mutated Citrullinated Vimentin Are a Better Predictor of Disease Activity at 24 Months in Early Rheumatoid Arthritis Than Antibodies Against Cyclic Citrullinated Peptides. J Rheumatol 2008;35:1002–8.
12. Larsen A. Radiological grading of rheumatoid arthritis. An interobserver study. Scand J Rheumatol 1973;2:136–8.
13. Plenge RM, Seielstad M, Padyukov L, et al. TRAF1–C5 as a Risk Locus for Rheumatoid Arthritis — A Genomewide Study. N Engl J Med 2007;357:1199–209.
14. Choi HK, Hernán MA, Seeger JD, et al. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. The Lancet 2002;359:1173–7.
15. Wolfe F, Michaud K. The National Data Bank for rheumatic diseases: a multi-registry rheumatic disease data bank. Rheumatology 2011;50:16–24.
16. Trynka G, Hunt KA, Bockett NA, et al. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. Nat Genet 2011;43:1193–201.
17. Duerr RH, Taylor KD, Brant SR, et al. A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. Science 2006;314:1461–3.
18. Knevel R, Tsonaka R, le Cessie S, et al. Comparison of methodologies for analysing the progression of joint destruction in rheumatoid arthritis. Scand J Rheumatol 2013;42:182–9.
19. Van der Woude D, Young A, Jayakumar K, et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug–free remission in rheumatoid arthritis: Results from two large early arthritis cohorts. Arthritis Rheum 2009;60:2262–71.
20. James D, Young A, Kulinskaya E, et al. Orthopaedic intervention in early rheumatoid arthritis. Occurrence and predictive factors in an inception cohort of 1064 patients followed for 5 years. Rheumatology 2004;43:369–76.
21. Humphreys JH, Verstappen SM, Hyrich KL, et al. The incidence of rheumatoid arthritis in the UK: comparisons using the 2010 ACR/EULAR classification criteria and the 1987 ACR classification criteria. Results from the Norfolk Arthritis Register. Ann Rheum Dis 2013;72:1315–20