The Role of Rheumatoid Arthritis Genetic Susceptibility Markers in the Prediction of Erosive Disease

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Abstract

The need to treat rheumatoid arthritis patients aggressively early in the disease course to avoid permanent radiological damage may result in overtreating a proportion of patients who would have achieved low disease activity with minimal treatment. The prediction of disease course and severity at diagnosis would therefore allow the adaptation of treatment regimes to specific patient needs. First, we review briefly advances made in the identification of non-genetic markers of erosive disease. Secondly, we present a review of the literature on the role of human leukocyte antigen (HLA)-DRB1 rheumatoid arthritis susceptibility alleles in predicting erosive disease or the extent of radiological damage. Finally, studies of non-HLA rheumatoid arthritis susceptibility single nucleotide polymorphisms as putative markers of radiological destruction are reviewed. Currently, no demographic, clinical, laboratory or genetic predictor of disease severity can be reliably used to guide clinical decisions. We highlight specific methodological issues inherent to genetic severity studies and suggest avenues for future research.

Keywords

Genetic markers, rheumatoid arthritis, severity, susceptibility, erosive disease, personalised medicine

Disclosure: The authors have no conflicts of interest to declare.

Acknowledgements: Sebastien Viatte is supported by a research grant from the Swiss Foundation for Medical-Biological Scholarships (SSMBS), managed by the Swiss National Science Foundation (grant reference number PASMP3_134380). This grant is financed by a donation of Novartis to the SSMBS.

Received: 23 March 2012 **Accepted:** 13 April 2012 **Citation:** *European Musculoskeletal Review*, 2012;7(2):102–7

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Rheumatoid arthritis (RA) is characterised by inflammation of synovial joints and its destructive course accounts for high disability and impaired quality of life in adults; in addition to the patients' suffering, the economic burden, in terms of healthcare and societal costs, is high.[1,2] Early and aggressive treatment has been shown to result in better clinical, radiological and economic outcomes.[3–7] If the 'therapeutic window of opportunity' is missed, irreversible damage might occur and disease control or even remission will be more difficult to achieve.[8] The current guidelines, therefore, advise early initiation of treatment for every patient with suspected RA. However, data from cohorts of early inflammatory polyarthritis (defined as swelling of two or more joints lasting for four or more weeks), where patients have been followed prospectively and which were established long before the introduction of the concepts of and guidelines for early intervention, suggest that a large proportion of patients with early inflammatory arthritis will achieve low disease activity without any disability or erosive disease without treatment (see *Table 1*). Indeed, even in those who go on to satisfy 1987 American College of Rheumatology (ACR) criteria for RA, a significant minority (42 % in the example shown in *Table 1*) remain non-erosive by five years. Therefore, in certain, yet unidentified patient groups, overtreatment might increase the patient burden more than the disease would do without treatment. Hence, there is undoubtedly the need to identify those patients for whom early and aggressive treatment will be beneficial (who can then receive the step-down approach to therapy), and patients for whom late and cautious treatment initiation will be suitable (step-up approach). In other words, early prediction of disease severity is crucial to achieve optimal patient care.

Disease outcome is frequently assessed by the extent of radiological damage in the hands and feet for several reasons.

- Radiological damage is an objective and quantitative measure based on X-rays and consequently has a lower variance than functionality, as defined by the health assessment questionnaire (HAQ) score.
- Functionality is correlated in the long term with joint damage.[9]
- Radiological damage in the hands and feet is representative of the overall structural damage in a patient.[10,11]

The most common methods to quantify radiological damage are the Sharp score, the Larsen score and their modifications.[12–18]

Non-genetic Markers of Erosive Disease

Many studies have been conducted to identify demographic, clinical or biological factors predisposing to erosive disease or radiological progression and have been reviewed by Harrison and Symmons in 2000[19] and more recently by Markatseli et al.[20] In summary, age does not seem to be reliably correlated with outcome, while gender might

be. Several studies report a poorer outcome in females, but findings are not always consistent.[19–22] The association between gender or age and disease severity seems therefore much weaker than observed with disease susceptibility. Disease duration, time-averaged or baseline measures of swollen joint count (SJC), erythrocyte sedimentation rate (ESR), time-averaged measures of C-reactive protein (CRP), rheumatoid factor (RF) positivity, anti-citrullinated protein antibody (ACPA) positivity, presence of erosions and radiographic score at baseline are recognised predictive factors of radiographic damage.[20–25] Although physicians work mainly with the Disease Activity Score (DAS) and rarely with its components individually, DAS as a predictor of disease severity has been evaluated in only few studies (see *Table 2*).[9,20] Overall, the presence of anti-cyclic citrullinated peptide (CCP) antibodies at baseline is the strongest predictor for erosions at baseline (odds ratio [OR] = 2.5) and at five years (OR = 10.2).[26] Seroconversion precedes disease onset and anti-CCP status is therefore an early marker as well.[27] Nevertheless, a high proportion (27 %) of anti-CCP negative patients develop erosions by five years,[26] indicating that anti-CCP antibodies alone are not a sufficiently accurate marker to guide clinical treatment decisions.

Many more non-genetic parameters have been evaluated as putative predictors of outcome, in particular of erosive disease; however, the results of these studies lack consistency, possibly in part due to differences in study design and study populations.[19,20] Moreover, even if several consistent prognostic laboratory markers are combined, together they still only explain less than 50 % of the total variance of joint damage (*Table 3* and reference 28). Therefore, to date, no predictor of severity is sufficiently accurate for use in daily clinical practice to guide treatment decisions.

Table 1: Early 'Aggressive' Treatment and Rheumatoid Arthritis Outcome – Unpublished Observations from the Norfolk Arthritis Register

Erosions at Year 5	Early Long-term Treatment		
	No	Yes	Total
No	257	166	423
Yes	87	270	357
Total	344	436	780

The table above allocates 780 patients with early inflammatory polyarthritis into two different treatment and severity categories. Only 56 % of patients received an early long-term treatment; of those, 91 % satisfied 1987 ACR criteria for RA by five years (data not shown) and 62 % developed erosive disease by five years. Forty-four percent of studied patients did not receive early long-term treatment. Of those, only 54 % developed RA by five years (data not shown) and 87 out of 344 (25 %) developed erosive disease by then. The initiation of early long-term treatment is strongly predictive of erosions in this setting (odds ratio 4.8; 95 % confidence interval [CI] 3.5–6.6; p=4.0x10^{-23}). This association is independent of baseline anti-CCP status (multivariate analysis, data not shown). This should not be interpreted as treatment exacerbating disease severity. Instead, physicians, based on their own clinical evaluation of the patient's condition, can accurately identify at baseline those patients who will develop erosive disease five years later, and treat them early. The physician's prediction of severity is independent of anti-CCP status, the best available predictor of disease severity today.

The Norfolk Arthritis Register (NOAR) is a prospective cohort of patients with inflammatory polyarthritis (IP),[1] which has been recruiting patients since 1989.
Early treatment is defined as treatment initiation with a second-line drug (i.e. steroid, any disease-modifying anti-rheumatic drug [DMARD] or any biologics) within less than six months of symptom onset. Long-term treatment is defined as cumulative treatment duration with any second-line therapy of at least six months within the first five years of follow-up. As an example, a patient treated for two months with prednisone at disease onset and then for five months with methotrexate during the third year of follow-up will be classified in the category 'long-term treatment'. NB This classification based on 'treatment quantity', differs from the standard definition of 'aggressive treatment', where the number of drugs used is important. Early long-term treatment: 'Yes' defined as 'Early treatment' and/or 'Long-term treatment'. 'No' defined as neither 'Early treatment' nor 'Long-term treatment'. Methods: 4,369 patients were recruited between 1989 and 2010. Patients with non-missing data concerning erosion status at year five, the date of treatment initiation, treatment type and duration were included in the analysis. Logistic regression was performed using with Stata version 10.1 (Stata Statistical Software: Release 10; Stata Corp., College Station, TX).
RA = rheumatoid arthritis; ACR = American College of Rheumatology; CCP = cyclic citrullinated peptide.

Genetic Markers of Erosive Disease

The failure of clinical or biological markers to accurately identify patients at risk of developing a severe disease course justified the search for genetic markers of erosive disease. Genetic markers present the advantages of being objective parameters, easily measured, present before disease onset, that do not change over time and that are not influenced by external factors, such as treatment. However, it is *a priori* not obvious that disease severity is genetically determined.

RA is a multifactorial disease and is thought to arise from a combination of genetic and non-genetic factors. The contribution of genetic factors to RA susceptibility, the heritability of RA, has been estimated to be approximately 50–60 % in twin studies,[29] which is similar for anti-CCP positive and anti-CCP negative RA.[30] Twin studies to investigate the contribution of genetic factors to RA severity in humans are difficult to conduct. A single twin study has been reported, to date, to address this question.[31] Though only 20 monozygotic twins, eight dizygotic twins and 40 unrelated RA patient pairs were analysed, the variation in joint damage was highest in unrelated patient pairs, followed by dizygotic twins and was smallest between monozygotic twins, indicating a genetic contribution to radiologic joint destruction in RA. The heritability could not be accurately calculated due to the small sample size. A recent study performed in Iceland evaluated the heritability of the severity of joint destruction using two different methods. The first one, based on a comprehensive genealogy database, estimated heritability to be 45 %, while the other technique, based on identical-by-descent (IBD) calculations and genome-wide genotyping, estimated heritability to be 58 %.[32] Therefore, it seems that the genetic contribution to susceptibility and severity of RA is similar.

Several candidate gene studies have been performed to identify non-human leukocyte antigen (HLA) genetic markers of RA severity and have been recently reviewed by Marinou et al.[33] The main focus of interest in recent years has been on cytokines and interleukins such as interleukin 1α (*IL-1α*), *IL-1β, IL-2, IL-4, IL-6, IL-10*, tumour necrosis factor α (*TNF-α*), transforming growth factor β (*TGF-β*) or matrix metalloproteinases (MMPs) such as *MMP-1* and *MMP-3*. However, many of these studies were underpowered and associations have not been independently replicated, so none of the above-mentioned polymorphisms are confirmed markers of erosive disease. A second focus of investigation has been RA susceptibility loci, including HLA markers, as putative predictors of RA severity. The current review will focus on the latter, which is discussed in the following sections.

Susceptibility Markers as Severity Markers

The number of genetic markers confirmed to be associated with RA at genome-wide levels of significance (<5x10^{-8}) (susceptibility polymorphisms) has steadily increased in the last few years, culminating in 2010 in a large meta-analysis by Stahl et al.,[34] which reported 31 confirmed HLA and non-HLA susceptibility loci. It is still a matter of debate as to whether the identified RA susceptibility single nucleotide polymorphisms (SNPs) are only predisposing to disease, or if some predispose to disease severity as well, as many of the studies analysed samples from RA patients with severe disease.

Human Leukocyte Antigen Markers

The largest genetic risk for developing RA arises from a group of alleles of the HLA DRB1 gene, collectively referred to as the shared epitope (SE).[35] Carriage of the HLA-DRB1 SE alleles has been shown convincingly

Table 2: Disease Activity Score 28 (DAS28) at Baseline or its Components as Predictors of Erosive Disease at Year Five; Unpublished Observations from the Norfolk Arthritis Register

	OR	95 % CI	p-value
TJC28	1.01	0.99; 1.03	0.16
SJC28	1.06	1.04; 1.09	7.4E-08
CRP	1.01	1.01; 1.02	1.2E-06
DAS28	1.40	1.24; 1.55	2.7E-09

Many studies have investigated the association between individual DAS28 components and erosive disease, but few have tested DAS28 itself or compared it with its components. This table presents the results of univariate logistic regression analyses performed in the NOAR register. SJC28 and CRP at baseline are associated with disease outcome at five years, but not TJC28. This is in line with published observations. This could be explained by a larger inter-patient variance in TJC28 due to the patient's subjective evaluation of pain, while SJC28 is a more objective measure. Since the scales used to measure DAS28 and its components are different, the odds ratio (OR) cannot be compared. The analysis presented here was performed in over 800 patients with inflammatory polyarthritis from the NOAR register (see Table 1). Dependent categorical variable: erosion status at year 5. Independent variable: either of the components at baseline of the DAS28 or DAS28 itself at baseline.
*TJC28 = tender joint count (28 joints); SJC28 = swollen joint count (28 joints); CRP = C-reactive protein at baseline; DAS28 = Disease Activity Score (DAS)-CRP on 28 joints, according to www.das-score.nl/: [0.56*sqrt(TJC28) + 0.28*sqrt(SJC28) + 0.36*ln(CRP+1)] * 1.10 + 1.15, where sqrt is the square root and in the natural logarithm.*

to be associated with radiological damage across many independent studies.[36–41] In particular, the HLA-DRB1*0401/0404 combination shows an increased association with early disease onset and severity.[42] The influence of HLA-DRB1 on susceptibility to and severity of rheumatoid arthritis was reviewed by Gonzalez-Gay et al. in 2002.[41] In 2004, a large meta-analysis of 29 studies and 3,240 patients demonstrated a significant association of the SE with erosions (OR = 2.0; 95 % confidence interval [CI] = 1.8–2.2).[43] Important differences in effect size (OR) were shown between different ethnic groups, possibly due to different SE allele frequencies between populations. HLA-DRB1*0401 was the strongest predisposing allele for erosive RA among northern European Caucasians, suggesting that susceptibility markers could be severity markers as well.

The Tezenas du Montcel classification in 2005 of HLA-DRB1 alleles according to their amino acid sequence at position 70–74 of the third hypervariable region of the HLA-DRβ chain[44] showed a more differentiated picture. The KRAA amino acid pattern at positions 71–74 defines the so-called HLA-DRB1 S_2 alleles –including HLA-DRB1*0401, and has been confirmed to be associated with more severe radiological damage with a gene–dose effect.[45,46] It is interesting to note that radiological damage was defined differently in the two studies: in the first, the outcome of radiologic progression was calculated by subtracting the baseline radiographic damage score (Sharp/van der Heijde) from the four-year follow-up score; in the second, the outcome was the absolute Larsen score, as measured at different time-points for different patients. In that study, regression analysis was performed with adjustment for disease duration; regression analysis assumes a linear relationship between Larsen score and disease duration, which has been shown not to be the case.[9] Although these two studies identified the S_2 alleles as risk alleles for radiological damage, they were inconsistent about whether protective alleles were also present and which they were.

There have been several reports about different HLA DRB1 protective alleles associating with radiological damage in RA: the DRRAA amino acid motif was associated in the study by Gourraud et al.[45] while two studies found association with the DERAA motif.[46,47] A large European meta-analysis[48] of susceptibility studies, however, did not support any of the previous classifications of protective alleles and showed that the protective effect on susceptibility of the allele classifications based on the DERAA sequence was no longer present after exclusion of DRB1*13. The study indicates that protection against ACPA-positive RA is predominantly associated with HLA-DRB1*1301. Whether the same is true for severity is unknown.

Interestingly, the pattern of association between SE alleles and RA susceptibility and severity shows differences. First, the effect size and strength of association are higher for disease susceptibility than is usually observed for disease severity. However, no formal comparison has been undertaken within a single study to address this issue. Moreover, the effect size of susceptibility risk alleles varies greatly between alleles, a fact that renders a comparison between susceptibility and severity difficult. Secondly, it seems that the relative contribution of HLA-DRB1 alleles to susceptibility and severity could be different: the susceptibility risk hierarchy of SE alleles according to the Tezenas du Montcel classification is different to the severity hierarchy.[45,46] Finally, protective alleles could be different between susceptibility and severity.[45,46,48] However, these three statements are based on comparisons between single studies, underpowered to address the issues, and require validation in larger cohorts. The SE is strongly associated with anti-CCP status,[46,49] which is in turn strongly associated with erosive disease.[26] It has been suggested that the SE plays a pivotal role in the production of ACPA and that ACPA are pathogenic, but it is still a matter of debate today as to whether the association between SE and erosive disease is independent of the ACPA status or not.[50,51]

The study of the relationship between the SE and RA susceptibility or severity in the last two decades has highlighted several issues, useful for the search of non-HLA markers of severity. First of all, the definition of the outcome variable is more complicated for severity than it is for susceptibility. Although radiological severity is a more objective measure than functional severity, there are several ways to define it. Severity can be defined categorically as the presence or absence of erosions, to determine the 'susceptibility to erosive damage', or as a radiological score above or below the median. Severity can be a continuous variable, like the number of eroded joints or a radiological score. Several radiological scoring methods have been developed but the modified Sharp and Larsen scoring systems are the ones most commonly used. Studies in which severity is defined as a continuous score have been performed either in the entire patient population, or sometimes only in patients with erosions, to determine the 'severity of erosive damage'. Radiological damage increases non-linearly over time, so that the results of cross-sectional studies performed at different disease durations might differ. Secondly, studies of small sample size (fewer than 100 cases and 100 controls) were sufficient initially to unequivocally establish the role of the SE as a susceptibility marker. It was more challenging to establish its role in disease severity, and the identification of SE subcategories associated either with risk or protection against severe forms of RA requires much larger sample sizes and replication has still not been achieved in several instances. Thirdly, confounders are different for susceptibility analysis and severity analysis (for example, age, gender, smoking, treatment). As shown in *Table 1*, treatment is a predictor of radiological damage (the patients with the most severe disease are the ones who receive more aggressive therapy earlier in disease course), but treatment prevents the development of erosions as well, which are statistically two opposite effects, of different effect size. This makes it difficult to account for treatment effects in analyses, and most studies simply do not adjust for treatment at all. Finally, although

the SE confers the highest risk for RA susceptibility, it has not been possible to clearly answer the question as to whether HLA susceptibility loci are always severity loci as well.

Interestingly, the above-mentioned considerations are only valid for anti-CCP-positive RA; the association between SE alleles and susceptibility to anti-CCP-negative RA is still a matter of controversy, with inconsistent findings reported in the literature.[48,50,52,53] Data on genetic predictors of radiological damage in anti-CCP-negative RA are very scarce.

Taken alone or even together with other confirmed markers of severity, the SE explains only a small proportion of the total variance of the Larsen score (see *Table 3*), which motivated researchers to hypothesise that non-HLA RA susceptibility SNPs would be predictors of severity as well.

Table 3: Percentage of the Variance in Larsen Score at Year Five Explained by Severity Predictors. Unpublished Observations from the Norfolk Arthritis Register

Association with Larsen at Year 5	P-value	% Var
SE	8.9E–08	5.9
SJC28	1.5E–04	4.3
CRP	1.2E–04	10.0
DAS28	1.9E–04	4.1
Anti-CCP	4.2E–22	25.0
DAS28, anti-CCP, SE	1.6E–21	26.8
SJC28, CRP, anti-CCP	1.7E–23	30.7
SJC28, CRP, anti-CCP, SE	4.9E–22	31.1

Table 3 compares the contribution of individual predictors to the total variance of radiologic damage: CRP alone at baseline is the best, though very modest, predictor and is better than DAS28. DAS28 does not perform better than SJC28 alone. A confirmed and statistically highly significant predictor therefore does not mean a good predictor. Multivariate regression analysis (data not shown) of the three individual DAS28 components does not affect their effect size and confirms an independent association of CRP and SJC28, while TJC28 remains unassociated. The association of confirmed predictors of radiological outcome or DAS28 with Larsen score at year five was tested either in univariate or multivariate analysis. The p-value for the model is reported, as well as the percentage of the variance (% var) of the Larsen score explained by the predictors, which are all measured at baseline. The inclusion of the SE in the model does not improve much of the variance explained, justifying the search for other genetic predictors.
Over 500 patients with inflammatory polyarthritis of the NOAR register (see Table 1) had available data for the multivariate analysis, while over 800 patients were available for some univariate analyses. See Table 2 for further abbreviations. SE was coded as 0, 1 or 2, reflecting the number of copies of the shared epitope carried by a patient. To account for a non-normal distribution of the Larsen score at year five, zero-inflated negative binomial regression was applied to test for association between independent variables and the outcome. The percentage of variance explained by the model was calculated as the square of the correlation coefficient between observed and predicted values of the Larsen score, but not corrected for the number of variables included in the model. SE was defined as the following HLA-DRB1 alleles: "0101" "0102" "0104" "0105" "0107" "0108" "0110" "0111" "0401" "0404" "0405" "0408" "0409" "0410" "0413" "0416" "0419" "0421" "0423" "0426" "0428" "0429" "0430" "0433" "0434" "0435" "0438" "0440" "0442" "0443" "0445" "1001" "1113" "1126" "1134" "1402" "1409" "1413" "1417" "1419" "1420" "1421" "1429" "1430" "1431" "1432" "1434" "1441" "1446" "1447" "1448".
CCP = cyclic citrullinted peptide; CRP = C-reactive protein; DAS28 = Disease Activity Score in 28 joints; SJC28 = swollen joint count.

Non-HLA Markers

After the SE, the 1858C->T (rs2476601) SNP at the *PTPN22* locus confers the largest genetic risk for developing RA. This *PTPN22* SNP was shown to be significantly associated with the annual progression rate of the Sharp–van der Heijde score in a 10-year longitudinal study in Norwegian patients,[54] and marginally associated with the Larsen score in a UK cohort,[55] while no association was found between the same SNP and erosions in patients from the US;[56] between a perfect proxy (rs6679677) and the Sharp–van der Heijde score in a six-year longitudinal study in the Netherlands;[57] or between any SNP at the *PTPN22* locus with either erosive status, Larsen score at year five or other markers of clinical outcome in a cohort of inflammatory polyarthritis in the UK.[58] These conflicting results could be explained by a relative lack of power in certain studies and/or differences in allele frequencies, cohort characteristics and definition of the outcome.

Other confirmed RA susceptibility SNPs have been tested as putative markers for disease outcome. In one study,[56] cytotoxic T-lymphocyte antigen 4 (*CTLA-4*) (rs3087243) was found not to be associated with erosive phenotype.

Scherer et al.[59] investigated five SNPs in low linkage disequilibrium (LD) spanning an RA susceptibility region at 6q23, near the *TNFAIP3* gene: rs1878658, rs675520, rs9376293, rs10499194 and rs6920220. Two of these SNPs (rs10499194 and rs6920220) and an additional one, rs5029937, have been confirmed to be independently associated with RA susceptibility in ACPA-positive patients.[34,60] rs675520 and rs9376293, but not the two confirmed RA susceptibility SNPs, were found to be significantly associated with the Sharp–van der Heijde score in ACPA-positive patients in this five-year longitudinal study. None of the five tested SNPs were associated with radiologic damage in ACPA-negative patients. No replication study was performed. The discrepancy between SNPs associated with susceptibility and those with severity could indicate that the causal variants might not have been identified yet and that further fine mapping experiments in well powered studies, including concurrent analysis of susceptibility and severity, with subsequent replication, are required. Alternatively, it is plausible that the pathophysiological mechanism conferring susceptibility is different to that conferring severity, but involves the same genetic regions.

Van der Linden et al. investigated the role of SNPs at five confirmed RA susceptibility loci (*CD40, KIF5A, CCL21, PRKCQ* and *TNFRSF14*) plus *CDK6* in the rate of radiologic joint destruction using a longitudinal cohort of 563 RA patients from the Netherlands. *CD40* and *CDK6* polymorphisms were found to be associated with the Sharp–van der Heijde score in ACPA-positive patients, but only the *CD40* SNP was replicated in an American cohort. Surprisingly, carriage of the susceptibility risk allele (major G allele of *CD40* rs4810485) was associated with a lower rate of joint destruction in both cohorts.

Kurreeman et al.[61] identified the *TRAF1/C5* locus, in particular rs10818488, as a marker of RA susceptibility and severity, as defined by the progression of the Sharp–van der Heijde score over two years. The association with radiological damage, as defined by the presence of erosions, was replicated in an independent cohort by Plant et al.[62] Furthermore, testing in a small number of patients from Egypt also found an association with *TRAF1/C5* polymorphisms and RA severity.[63]

The most comprehensive and systematic study assessing the role of RA genetic susceptibility markers in the prediction of erosive disease in patients with recent-onset inflammatory polyarthritis (IP) or RA was performed recently by Plant et al.[62] Eighteen SNP markers from 14 loci *(AFF3, CCL21, CD226, CD40, CTLA4, IL2/IL21, IL2RA, IL2RB, IL7R, KIF5A, PRKCQ, STAT4, TNFAIP3, TRAF1/C5*) were tested in a cohort of 1,049 patients for association with the presence of erosions cross-sectionally at year one and five ('susceptibility to erosive damage') and for association with Larsen score in patients with erosive disease ('severity of erosive damage'). None of the SNPs tested was associated with Larsen score, and only two SNPs, rs2900180 and rs10760130 (LD r^2 = 1

with rs10818488) at the *TRAF1/C5* locus showed association with erosions, independently of ACPA status. Interestingly, no association with radiological damage was found for *CD40* and *TNFAIP3*, which could be explained by a lack of power or absence of true associations at those loci.

Therefore, apart from associations at *TRAF1/C5* and *CD40*, no association of SNPs located at non-HLA RA susceptibility loci has been replicated in independent studies and therefore convincingly shown to be markers of erosive disease. The SNP marker at *CD40*, though replicated in an independent cohort, was protective against the development of RA, while being a risk factor for RA severity. Reports for *PTPN22* have been conflicting. Undoubtedly, replication experiments in well-powered studies are required to address the role of RA susceptibility loci in disease severity.

Conclusions

The need to predict RA severity for the initiation of early aggressive treatment in patients susceptible to developing disabling disease has been the driving motivation for the identification of markers of RA severity. Although anti-CCP status is strongly associated with erosive disease, its predictive value is still too low to guide clinical decisions. Composite scores, integrating demographic, clinical and laboratory markers into account, do not perform much better.

Genetic markers present the advantage of being objective and stable parameters and the association of HLA with RA has been known for 30 years. Much effort has therefore been invested in unravelling the complex relationship between HLA DRB1 and RA susceptibility and severity. It is clear that the SE is convincingly associated with RA severity but the independent contribution over and above anti-CCP status remains modest. Studies performed with the SE highlighted several methodological difficulties inherent to the identification of genetic markers of severity. First, there is currently no consensus on the most appropriate way to define radiologic damage, which makes it difficult to compare different studies. Moreover, population characteristics differ in terms of severity between different studies (cohort of early IP versus hospital-based cross-sectional studies). An additional aspect that makes studies difficult to compare and therefore to reproduce results is the strong and non-linear association between disease duration and severity. Finally, as has been shown by severity studies performed for SE alleles, large sample sizes are required to reliably identify and replicate markers of severity as well. Therefore, large cohorts of early RA patients followed prospectively with multiple X-ray records over time are required to identify and replicate markers of erosive disease. Few cohorts fulfilling those criteria are currently available.

The numerous studies performed with the SE and the few studies performed with non-HLA RA susceptibility SNP markers have shown that susceptibility markers cannot automatically be considered as severity markers. Among non-HLA RA susceptibility markers, only *TRAF1/C5* has been confirmed to associate with radiological damage, while a marker at *CD40* shows inconsistent results in terms of susceptibility and severity.

Therefore, all susceptibility markers need to be further tested or replicated in well-powered studies to address their role as severity markers. Since markers of severity might well be different to susceptibility markers, a well-powered genome-wide association study to identify markers of RA severity is required. ■

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