PART 2

Genetic and serologic factors in predicting radiographic progression in rheumatoid arthritis

4

Biomarkers for radiographic progression in rheumatoid arthritis

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ABSTRACT

Treatment of patients with rheumatoid arthritis (RA) is rarely personalized, since predictors of disease course are lacking. The severity of RA can be measured objectively by radiographic progression. The most reliable way to measure radiographic progression is in a longitudinal cohort with serial time points, scoring on a quantitative scale, with a validated scoring method and trained readers.

Current models used to predict radiographic progression are based on C-reactive protein and anti-citrullinated protein antibodies. Other biomarkers could increase the prognostic ability of these models. In this review, we evaluated the published (and partly not published) data on genetic, serologic, and imaging biomarkers for the severity of joint destruction in RA.

We evaluated variants in 10 genes (*CD40*, *IL2RA*, *IL4R*, *IL15*, *OPG*, *DKK1*, *SOST*, *GRZB*, *MMP9*, and *SPAG16*). In 5 variants (*IL2RA*, *DKK1*, *GRZB*, *MMP9*, and *SPAG16*), we found evidence of an association at the functional level. We evaluated several serological biomarkers, namely, autoantibodies (RF, ACPA, anti-CarP), markers related to inflammation (ESR, CRP), and proteinases or components of the extracellular matrix of bone and cartilage (MMP3, CTX-I, CTX-II, COMP, TIMP1, PYD, RANKL/OPG, CXCL13). Finally, we evaluated markers that can be visualized by ultrasound or MRI, including erosions, bone marrow edema, synovitis, and tenosynovitis. Several studies showed that bone marrow edema and synovitis on MRI are robust predictors of radiographic progression. Some studies showed that inflammation detected with ultrasound predicted radiographic progression.

Future studies will reveal whether adding and combining all these different biomarkers will increase the accuracy of risk models predicting radiographic progression in RA.

INTRODUCTION

Providing personalized medicine in rheumatoid arthritis (RA) is one of the great challenges for the near future. In order to achieve this goal, it is necessary to fulfil 2 conditions: adequate estimation of severity so that the patients who develop severe disease can be differentiated from patients with mild disease; and identification of an individual patient's responsiveness to specific disease-modifying anti-rheumatic drugs (DMARDs) or biologics. The present review focuses on the first issue: predictors of the severity of the course of RA.

Measurement of the severity of RA

Severe RA is not uniformly defined. Patients often refer to the degree of pain or fatigue and the ability to perform daily activities and work. Rheumatologists are more concerned with the level of inflammation (expressed by the number of inflamed joints), the level of acute phase reactants, and pooled severity indices such as disease activity scores. Scientists focus on outcome measures that can be assessed objectively, such as joint destruction and mortality.[1] These perspectives are essentially similar, since levels of impaired functionality, inflammation, and structural damage are correlated.[2] Fluctuations in disease activity are directly related to changes in radiographic progression, although this relationship is less present in specific treatments such as tumor necrosis factor (TNF) blockers.[3] Joint inflammation and joint destruction both play an independent role in impaired physical functioning.[4]

Deciding which measure of severity is best depends on the reason it is selected. The advantages and disadvantages of several severity measures are summarized in Table 1. For research purposes, the rate of joint damage, as visualized on radiographs of the hands and feet, is the most common outcome measure. The main advantage is that it can be assessed objectively using a validated scoring method. The radiographs can be scored by the same reader, thus making it possible to evaluate within-reader variation and between-reader variation (in the case of ≥1 reader). Another advantage of measuring the severity of joint damage is that it accumulates over time and thus reflects disease history. In summary, the rate of joint destruction correlates with the cumulative burden of inflammation over time, is highly linked with physical function and other outcomes such as work disability, and is inexpensive to measure using validated scoring methods; consequently, the rate of joint destruction is a comprehensive endpoint in observational studies. In this review, we focus on radiographic joint damage as an outcome for identifying new risk factors for severity of RA.

Measurement of the severity of radiographic damage in RA

The absence or presence of joint damage can be assessed based on "erosiveness" or "erosive disease". This qualitative method has 2 disadvantages. First, the degree of joint damage cannot be discerned. The fact that 70%-75% of patients with early RA developed

Table 1 Measures of severity of RA and advantages and disadvantages for the use as outcome measures in studies that aim to identify biomarkers

RA severity measures	Advantages (+) and disadvantages (–)
Disease course measures	
Tender or swollen joint count	– Does not reflect cumulative severity of RA
Lansbury articular index[a]	– Fluctuates over time
DAS	
AUC of DAS over time	+ Reliable if DAS is assessed ≥3 times a year
HAQ	+ Reflects disease activity – Influenced by age, disease duration, social and psychosocial factors
Extra-articular symptoms	– Frequency nowadays decreasing thanks to introduction of potent treatment strategies
Disease outcome measures	
Mortality	+ Objective measure – May be influenced by non-RA-related causes – No increased mortality risk observed in case of DAS-guided treatment[b]
DMARD-free sustained remission[c]	+ Most favourable outcome of RA as it approximates cure – Long follow-up necessary in order to achieve this outcome and to assure that remission is sustained
Rate of radiographic joint destruction	+ Objective and well validated, quantitative scoring methods available + Quality of scoring can be easily expressed + Reflects cumulative severity of RA + No fluctuation in time – Scoring is time and cost consuming (need a trained scorer) (and eventually taken extra radiographs besides normal clinical care)

[a]In the Lansbury articular index, the joint counts are weighted for the joint size. Such weighting may be preferable, as the volume of inflamed synovial tissue would be proportional to the serum CRP level and the level of disability.
b(111;112)
[c](113)
RA, rheumatoid arthritis; DAS, disease activity scale; AUC, area under the curve; HAQ, health assessment questionnaire; DMARD, disease-modifying antirheumatic drug.

erosions within the first years of follow-up indicates that this measure is suitable in very early phases of the disease, but not in more advanced stages.[5] Second, no uniform definition for erosiveness or erosive disease exists. Performing analyses with non-validated outcome measures increases inter-observer variation and variation between studies. Indeed, the various descriptions for erosive RA include any radiographic evidence of erosions,[6,7] a cortical break of ≥2 mm (8), and presence of ≥2 or ≥3 erosions.[9;10] According to the 2010 ACR/EULAR criteria, typical erosive disease is defined as at least 3 small hand or foot joints

with an interrupted cortex. This condition was associated with a specificity of >85% for prescription of methotrexate and >90% for disease persistency.[11]

Currently used quantitative scoring methods to measure joint damage severity include the Sharp van der Heijde score (SHS) and (modified) Larsen score. These quantitative measurements are more discriminative than binary outcome measures, have been validated, and can be used in all phases of the disease.[12,13] Both the SHS and Larsen score are used to assess the joints of the hands and feet. Of the two, SHS is the only one that differentiates between bone erosions and cartilage damage, which is visualized as joint space narrowing. Compared to the Larsen score, the SHS is more sensitive for detecting changes over time, although it is also more time-consuming to apply.[12]

Given that quantitative outcomes are more accurate than qualitative outcomes and repeated outcomes are even more accurate, the number of time points is a key element of optimal assessment of joint destruction. Within-patient correlation of serial measurements is important when scoring radiographs. Unlike damage on radiographs from different patients, the severity of damage on serial radiographs from the same patient is highly correlated. Compared to measuring radiographs at only 1 time point, scoring radiographs at subsequent time points substantially diminishes within-patient variability in joint damage. Consequently, the power of the study is increased, a smaller number of patients is needed to detect a difference, and phenotypic misclassification is reduced.[14] Hence, a more precise estimation of the rate of joint destruction facilitates differentiation between true effects and noise.

Although radiographs are scored with a quantitative scoring method, sometimes only 1 radiograph per patient is available and the time point at which the radiographs were made varies between patients. Since radiographs are made at different phases of the disease, scores cannot be easily compared. In order to estimate the effect of risk factors on joint damage in this circumstance, the estimated radiographic progression per year can be determined by dividing the total radiographic score by the disease duration at the time the radiograph was taken. This approach is limited in that it assumes that the baseline score was zero and includes both patients with early RA and patients with advanced RA, thus precluding comparison of estimated radiographic progression rate. The course of joint damage is frequently linear in the early years and slows down as the disease progresses.[15] Inaccurate estimation of disease duration has a larger impact on patients with short disease duration. Consequently, the estimated radiographic progression per year may be overestimated in patients with short disease duration. An extreme situation is that of a patient with a long disease duration in which the maximal level of joint destruction has been reached but no further destruction has evolved since then; consequently, the level of joint destruction remains unchanged during further follow-up. If the single radiography is taken during this period with a stable SHS score, the estimated annual progression decreases with every year of follow-up. Therefore, the use of the estimated annual progression is

restricted to a short follow-up duration and harbors a risk of phenotypic misclassification in advanced disease stages.

In conclusion, the rate of joint destruction in longitudinal studies should be assessed at serial time points on a quantitative scale using validated scoring methods and trained readers.

Analysis of the rate of joint destruction in longitudinal studies

Radiographic data are by definition skewed, as many patients have little progression and few patients have marked progression. This skewness makes it difficult to apply statistical tests, many of which presume a normal distribution. However, a normal distribution can frequently be achieved by log-transformation of the data. Given that several radiographic measurements may be made, it is beneficial to use a statistical method that takes advantage of within-patient correlations, as this will yield more precise estimations of the progression rate and, therefore, increase the possibility of detecting differences. Repeated analyses based on covariance matrices, for instance, linear mixed models, also make it possible to include patients with missing radiographs over time. This possibility is relevant, since missing data are often not completely random but related to the severity of the disease course; over time, patients with the most severe disease can die and those with the least severe disease are often lost to follow-up. Excluding these patients will make the obtained effect size less generalizable to the general patient population. In other words, including all patients in a specific population reduces the likelihood of selection bias that will be introduced if per protocol analysis is performed.

Treatment effects and other confounding factors

The effect of potential biomarkers on the rate of joint destruction should ideally evaluated in longitudinal cohorts of patients in whom the disease course of RA is natural (ie, untreated patients). Such may be the case of patients diagnosed and recruited in the 1980s or early 1990s, although such datasets are rare today. When studying more recently diagnosed patients, analyses should be adjusted for treatment effects to discern the role of the risk marker. Other potential confounding factors are age and gender. Whereas the effect of gender is different between cohorts, the effect of age is consistent: in almost all studies, older age at onset is associated with a more severe disease course.

BIOMARKERS

A biomarker is defined as a characteristic that can be objectively measured and evaluated as an indicator of a normal biological or pathogenic process; it may also be an indicator of response to a therapeutic intervention.[16]

According to this definition, the term biomarker covers any type of characteristic. Biomarkers are classified into 3 groups: genetic markers, serologic markers, and imaging markers.

An OMERACT task force proposed a much more stringent definition for a biomarker, namely, that it reflects structural damage in RA.[17] The definition is based on 14 requirements, which include not only criteria for the reliability of the assay and the discriminative ability of the marker, but also items reflecting the "truth". The criteria include the following: evidence that a biomarker reflects tissue remodeling demonstrated in animal models, immunohistochemical localization in joint tissue, and correlation between levels of the biomarker and scores of other surrogates for structural damage. These criteria were developed for serological markers but may also be applicable to other types of biomarkers. Nonetheless, this definition of a biomarker is more challenging to fulfill than the definition provided above. In this manuscript, we define biomarkers as genetic, serologic, and imaging markers that are predictive of radiographic progression in patients with RA.

Types of biomarkers for joint damage progression in RA

Twin studies have indicated that radiographic progression is in part heritable. Recent estimations on the Icelandic RA population yielded a heritability of 45%-58%.[18] The other 42%-55% can be explained by environmental or random factors. Genetic factors may predispose to serologic risk factors that are also predictive of the severity of joint damage and to joint damage via unknown mechanisms.

Serologic biomarkers are produced under the influence of both genetic and environmental factors, as illustrated by anti-citrullinated protein antibodies (ACPA). Their presence is associated with severe disease course, and certain *HLA-DRB1* alleles and smoking predispose to the development of these autoantibodies.[19]

Imaging characteristics can also act as biomarkers, as they can indicate a pathogenic process. Among the 3 categories of biomarkers discussed, serologic and imaging biomarkers are closest to the phenotype, because advanced imaging techniques actually visualize subclinical (and clinical) disease features (Figure 1).

GENETIC BIOMARKERS FOR THE SEVERITY OF RADIOGRAPHIC JOINT DESTRUCTION IN RA

Interest in the genetic background for susceptibility to RA has grown during recent years, and more than 40 predisposing genetic variants have been identified.[20] The vast majority were identified in genome-wide studies evaluating thousands of cases and controls.

Severity of RA is studied by making comparisons within patients, thus necessitating long-term longitudinal outcome data. Such datasets are scarce and consequently; studies

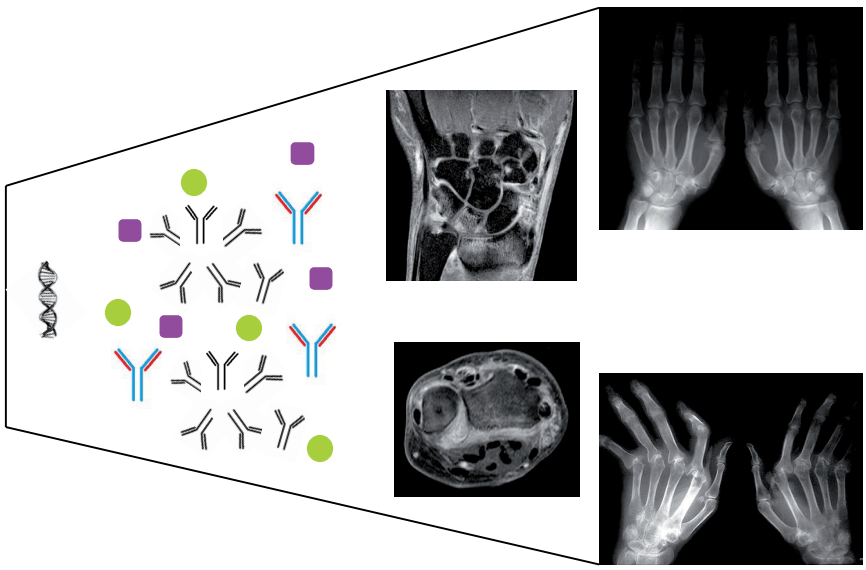

Figure 1 Illustration of different biomarkers of radiographic progression in RA; in general, the closer to the phenotype a marker is, the higher the effect size is
From left to right: genetic biomarkers (SNPs), serological biomarkers (auto-antibodies, acute-phase reactants, other markers related to inflammation or components of the bone or cartilage), imaging biomarkers (bone marrow edema, synovitis, tenosynovitis, and erosions visualized by MRI or ultrasound) and the outcome (radiographic progression or no radiographic progression). In this review, we observed that the effect increased from left to right, probably because the markers located at the right are more closely related to the phenotype.

with equal sizes as in the RA susceptibility field, are out of reach. Most genetic studies on progression of joint damage are relatively small. In addition, they were not performed with a hypothesis-free genome-wide approach, but rather investigated dedicated candidate genes.

It is difficult to know when a genetic association is real. Figure 2 depicts possible levels of evidence. The first level is the *P* value; in our view this level is insufficient to indicate whether a variant is true. In high-throughput studies, where many variants are typed and analyzed, *P* values $<5\times10^{-8}$ are generally considered to be valid. This cutoff is derived from a Bonferroni correction of 0.05 (alpha)/500,000 (number of single-nucleotide polymorphisms [SNPs] analyzed). If this number of SNPs is actually studied, the *P* value reflects a probability <5% that the finding is based on chance. However, the *P* value is also largely influenced by the number of subjects being studied. Hence, a *P* value obtained in a study including several thousands of subjects may still indicate a finding that is based on chance,

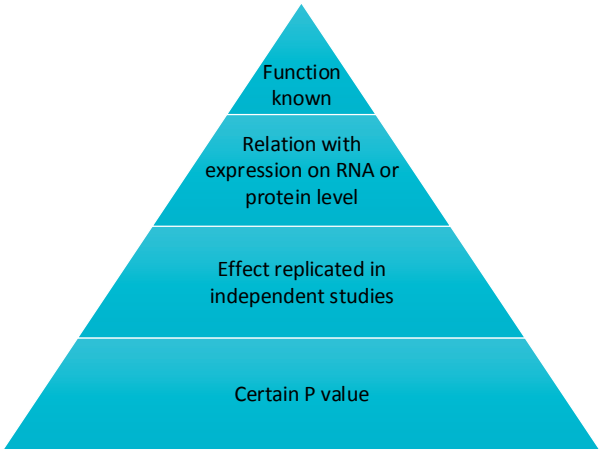

Figure 2 Level of evidence of data obtained in genetic studies; the higher up in the pyramid, the higher the level of evidence.

and a similar or higher *P* value obtained in a study containing several hundreds of patients may be indicative of a "true variant". In conclusion, using the *P* value as a measure to evaluate the reliability of findings is spurious. A more reliable method is replication. If a variant is statistically associated in several independent cohorts, the chance that the observation reflects chance finding is greatly reduced. Even more convincing are data that support the finding at a different level. Examples include genetic variants associated with changed expression at mRNA or protein level. Genetic associations that are not supported by expression data may also be genuine, although the presence of associations at the level of expression does increase reliability and may act as an initial step in better understanding the consequences of carrying a certain genetic variant. In the ideal situation, the pathway or mechanism via which a genetic risk factor influences disease is known.

During the last 2 decades, many studies have evaluated genetic markers in relation to the severity of RA. The results were often inconsistent, probably owing to small samples and different types of outcome measure.

We reviewed the literature and included unpublished data on genetic variants associated with progression of joint damage. We only reviewed genetic variants that were assessed in several populations. Variants that were assessed in only 1 cohort were not addressed, because of the low level of evidence, since the study was not replicated. The studies that were available measured joint damage in different ways, thus precluding a meta-analysis.

The candidate genes evaluated to date can be categorized as markers associated with the development of RA, markers of inflammation, and markers related to bone or cartilage.

Markers associated with the development of RA

HLA-DRB1

The *HLA-DRB1* alleles coding for the so-called shared epitope are the oldest genetic risk factor associated with progression of joint damage. This marker is also the most widely validated genetic risk factor. The presence of shared epitope alleles is a risk factor for ACPA, which are associated with progression of joint damage. Interestingly, in a recent study on progression in ACPA-negative RA, the *HLA* region was not identified as a marker for radiographic progression (manuscript under review), thus providing further evidence that the relevance of the *HLA-DRB1* region for progression lies in predisposition to development of autoantibodies. The *HLA-DRB1* region that codes for the shared epitope alleles can also code for the *DERAA* amino acid motif. The presence of this variant has been associated with protection against radiographic progression.[21] To our knowledge, this effect has never been studied in other cohorts and therefore has not been replicated. The mechanism by which *HLA-DRB1* alleles predispose to ACPA and progression of joint damage is not completely understood, although the hypothesis is that these genetic variants influence the immune response by affecting the antigen-binding site.

PTPN22

A coding variant in *PTPN22* (rs2476601) has been studied in 8 populations to establish an association with the severity of joint damage. Although the minor allele was associated with more severe progression in 2 studies,[22,23] subsequent studies in 5 other cohorts did not reveal a significant association (Table 1).[24-27] Performing a meta-analysis of these studies is difficult, owing to the different quantitative and qualitative outcome measures used.

TNFAIP3-OLIG3

Several variants in this region are associated with the risk of developing RA. rs6920220 was associated with progression of joint damage in a UK study consisting of 685 radiographs from 700 ACPA-positive RA patients.28 In a Dutch study consisting of 844 radiographs from 181 ACPA-positive RA patients, this association was not replicated,[29] and no association was present in unpublished data from several North American cohorts. (Table 2) Most available data are for rs675520, which was studied in 8 cohorts; a meta-analysis of 7 of these cohorts did not reveal a significant association with RA.[29,30]

C5-TRAF1

Although a significant association was observed for rs2900180 in the *C5-TRAF1* region in 2 UK cohorts,[31] other datasets did not reveal a significant association (Table 2). A meta-analysis of these data is required to draw conclusions on the relevance of this SNP for

progression of joint damage. An initial positive association was observed for rs10818488, which is another variant in this region; however, this association was not maintained in a meta-analysis of 7 cohorts.[30,32]

CD40

A genetic variant in *CD40* (rs4810485) was shown to be a risk factor for development of ACPA-positive RA. This marker was subsequently studied in relation to radiographic progression in 2 cohorts of ACPA-positive RA patients. A significant association was observed in both. Intriguingly, the minor allele that was associated with a decreased risk of RA was associated with more severe progression of joint damage. No ready explanation is available for this observation.[33]

IL2RA

The polymorphism rs2104286, which is found in the gene coding for the IL2 receptor α chain (*CD25*), is clearly associated with progression of joint damage. Significant associations were demonstrated in Dutch, Icelandic, and North American RA populations. Furthermore, the minor allele, which is associated with reduced progression, was also associated with lower serum levels of IL2RA, which correlated with progression.[34] A multivariate analysis including both *IL2RA* and serum IL2RA showed that only serum levels were independently associated with progression, suggesting that the genetic variant affects progression by a mechanism that also affects serum levels. rs2104286 is the only genetic variant predisposing to development and progression of RA for which functional data are available. Interestingly, this variant also predisposes to other autoimmune disorders such as multiple sclerosis and type 1 diabetes.

Variants in a further 2 susceptibility genes (*AFF3* rs11676922 and *BLK* rs13277113) have been studied in several cohorts, but no evident association with the severity of joint damage was found.

Inflammatory markers as candidates for joint damage progression

Joint damage results from deregulated inflammation or disturbances in bone or cartilage homeostasis (Figure 3). Joint destruction, as visualized on hand and foot radiographs, is the local loss of cartilage and bone resulting from inflammation. The presence of specific autoantibodies is thought to propagate the level and chronicity of inflammation and may directly and indirectly affect the level of structural damage.[35] Genes coding for inflammatory markers can also influence the level of inflammation and are therefore likely subjects for candidate gene studies focused on progression of joint damage in RA (Table 3).

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
HLA-DRB1	SE-alleles	324 RA	1301 radiographs (baseline, 1, 2, 3, and 4 years)	Modified SHS	Destructive	<0.05 (at all time points)	Van der Helm-van Mil et al.(21)
	SE*04	111 RA	At baseline and 1 and 2 years[b]	Modified Sharp: divided into 3 categories erosion gain	Destructive	0.02, 0.01, 0.05 (0, 1, and 2 years)	Goronzy et al.(114)
	SE-alleles	872 RA	827 radiographs (minimum disease duration of 3 years)	Modified LS	Destructive (only in RF–)	0.05	Mewar et al.(68)
	SE *04	87 RA	309 radiographs[c] (baseline, 1, 2, and 4 years)	LS	Destructive	0.01	Kaltenhauser et al.(66)
PTPN22	rs2476601 (+1858)	238 RA	At baseline, 1, 2, 5, and 10 years*	SHS	Destructive	0.01	Lie et al.(22)
	rs2476601 (+1858)	901 RA	901 radiographs[c] (at baseline)	Modified LS	Destructive	0.04	Marinou et al.(23)
	rs2476601 (+1858)	123 RA	At baseline, 1, 2, 4, and 6 years*	LS	Destructive	0.61, 0.06, 0.07, 0.14, 0.16 (0, 1, 2, 4, and 6 years)	Pierer et al.(25)
	rs2476601 (+1858)	302 RA	286 radiographs[c]	Erosive vs nonerosive	Destructive	0.20	Steer et al.(26)
	rs2476601 (+1858)	593 RA	1526 radiographs	SHS	Not reported	0.93	Van Nies et al.(27)
		397 ACPA+ RA	397 radiographs	SHS	Destructive	ACPA+ 0.22	
	rs2476601 (+1858)	689 RA	627 radiographs	Erosion present or absent	Destructive	0.2	Karlson et al.(24)

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
TNFAIP3-OLIG3/ 6q23	rs6920220	700 ACPA+ RA	685 radiographs (at baseline)	LS	Destructive	ACPA+ 0.02	Maxwell et al.(28)
	rs6920220	181 ACPA+ RA	844 radiographs (over 5 years)	SHS	Destructive	ACPA+ 0.76	Scherer et al.(29)
	rs6920220	600 RA (Leiden-EAC)	2846 radiographs (over 7 years)	SHS	Protective	0.53	Not published data.
		101 RA (Wichita)	337 radiographs (over 15 years)	SHS	Destructive	0.57	
		649 RA (NDB)	649 radiographs	SHS	Protective	0.25	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Destructive	0.97	
	rs675520	181 ACPA+ RA	849 radiographs (over 5 years)	SHS	Protective	ACPA+ 0.007	Scherer et al.(29)
	rs675520	2666 RA (meta-analysis of 7 cohorts)	6282 radiographs (4 repeated and 3 single measurement)	LS and SHS	Two protective and 5 destructive	0.49	Knevel et al.(115)

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
C5/TRAF1	rs2900180	445 RA	2161 radiographs (over 15 years)	LS	Destructive	0.011 (Combined data)	Viatte et al.(31)
		1446 inflammatory polyarthritis	2168 radiographs (baseline, 1 and 5 years)	LS			
	rs2900180	600 RA (Leiden-EAC)	2846 radiographs (over 7 years)	SHS	Destructive	0.056	Not published data.
		101 RA (Wichita)	337 radiographs (over 15 years)	SHS	Protective	0.988	
		649 RA (NDB)	649 radiographs	SHS	Destructive	0.371	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Protective	0.078	
	rs10818488	278 RA	556 radiographs (baseline and 2 years)	SHS	Destructive	0.008	Kurreeman et al.(32)
	rs10818488	2666 RA patients (meta-analysis of 7 cohorts)	6282 radiographs (4 repeated and 3 single measurements)	LS and SHS	Three protective and 4 destructive	0.72 (meta-analysis)	Knevel et al.(30)
CD40	rs4810485	250 ACPA+ RA	2940 radiographs (over a maximum of 9 years)	SHS	Destructive	0.003	Van der Linden et al.(33)
		393 ACPA+ RA	393 radiographs	SHS	Destructive	0.021	

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
IL2RA	rs2104286	1750 RA (meta-analysis of 4 cohorts)	4732 radiographs (2 repeated and 2 single measurements)	SHS	Three protective and 1 destructive	7.2*10^-4 (meta-analysis)	Knevel et al.(34)
AFF3	rs11676922	1750 RA (meta-analysis of 4 cohorts)	4732 radiographs (2 repeated and 2 single measurements)	SHS	Two protective and 2 destructive	0.08 (meta-analysis)	Knevel et al.(34)
BLK	rs13277113	1750 RA (meta-analysis of 4 cohorts)	4732 radiographs (2 repeated and 2 single measurements)	SHS	One protective and 3 destructive	0.11 (meta-analysis)	Knevel et al.(34)

[a]The direction of the effect size is presented, irrespective of the *P* value of the obtained result. Hence, the direction of nonsignificant findings was also presented.
[b]Number of radiographs not reported in manuscript.
[#]These data were not reported in the manuscript, but derived from the data presented in the text or tables.
[†]The minor alleles were different between the cohorts. Between brackets is the allele with the lowest frequency in the specific cohort.
ACPA, anti-citrullinated protein antibodies; AFF3, AF4/FMR2 family; BLK, B lymphocyte kinase; CD40, cluster of differentiation 40; C5/TRAF1, complement component 5/tumor necrosis factor receptor-associated factor 1; HLA-DRB1, human leukocyte antigens-DRB1; IL2RA, interleukin 2 receptor alpha, member 3; LS, Larsen score; Leiden-EAC, Leiden early arthritis clinic; NARAC, North American Rheumatoid Arthritis Consortium; NDB, National Data Bank (American cohort); SHS, Sharp van der Heijde score; PTPN22, protein tyrosine phosphatase, non-receptor type 22; RA, rheumatoid arthritis; SE-alleles, shared epitope-alleles; TNFAIP3-OLIG3, tumor necrosis factor, alpha-induced protein 3-oligodendrocyte transcription factor 3.

IL1

TNF-α and IL-1 are pro-inflammatory markers that are overexpressed in RA. However, while few studies have been performed on genetic variants in *TNFA*, several studies have addressed variants in *IL1*. The activity of IL-1 reflects the function of 2 molecules, IL-1α and IL-1ß. IL-1α is cell-bound and IL-1ß is a secreted cytokine. No associations have been reported between variants in *IL1A* and the severity of radiographic damage in RA. For *IL1B*, rs16944 was evaluated in 3 studies but no effect was observed.[23,36,37] Similarly, despite a significant association between rs1143634 in *IL1B* and both serum levels and joint destruction in a study of 297 patients and 273 radiographs,[38] further studies (2762 patients and 5956 radiographs) could not replicate this association (Table 3).[23,37,39] Based on these data, variants in *TNFA*, *IL1B*, and *IL1A* do not predispose to severe destructive RA.

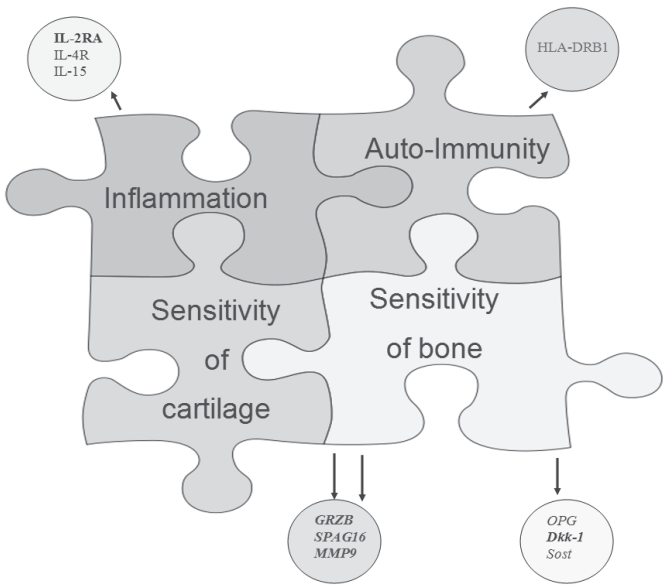

Figure 3 In RA, joint damage is the result of several processes: inflammation, auto-antibodies, and resistance of cartilage and bone against degradation.
The genetic markers that are replicated are presented; the genetic variants that are observed to be associated with differences at the level of expression are indicated in bold.
IL2RA, interleukin 2 receptor alpha; IL4R, interleukin 4 receptor; IL15, interleukin 15; HLA-DRB1, human leukocyte antigens-DRB1; GRZB, granzyme B; SPAG16, sperm associated antigen 16; MMP9, matrix metallopeptidase 9; OPG, osteoprotegerin; DKK1, dickkopf-related protein 1; SOST, sclerostin.

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity.

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL1B	rs16944 (–511)	712 RA	712 radiographs	Erosion vs no erosion	Protective	0.67	Johnsen et al.(37)
	rs16944 (–511)	932 RA	932 radiographs# (at baseline)	Modified LS	Not conclusive	0.8	Marinou et al.(23)
	rs16944 (–511)	157 RA	157 radiographs	LS plotted against disease duration	Protective	n.s.	Genevay et al.(36)
	rs1143634 (+3954)	297 RA	273 radiographs	destructive vs nondestructive acc. to wrist LS (>2 vs <2)	Destructive	0.03 (Larsen index 0.005) (HLA: 0.0001)	Buchs et al.(38)
	rs1143634 (+3954)	712 RA	712 radiographs	Erosion vs no erosion	Protective	0.54	Johnsen et al.(37)
	rs1143634 (+3954)	147 RA	147 radiographs	Right Larsen wrist score >=2 vs <2	NR	NS	Marotte et al.(39)
	rs1143634 (+3954)	880 RA	880 radiographs# (at baseline)	Modified LS	Not conclusive	0.1	Marinou et al.(23)
	rs1143634 (+3954)	600 RA (Leiden-EAC)	2846 radiographs (during 7 years)	SHS	Protective	0.07	Not published data.
		101 RA (Wichita)	337 radiographs (during 15 years)	SHS	Protective	0.62	
		649 RA (NDB)	649 radiographs	SHS	Destructive	0.77	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Destructive	0.25	

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL4	rs2243263	965 RA	892 radiographs (minimum disease duration of 3 years)	Modified LS	Not conclusive	0.3	Marinou et al.(42)
	rs2243263	600 RA	2846 radiographs (during 7 years)	SHS	NR	NS	Krabben et al. (41)
IL4R	rs1805010 (I50V)	302 RA	302 radiographs (2 years after disease onset)	Erosion vs no erosion	Destructive	<0.0001	Prots et al.(40)
	rs1805010 (I50V)	965 RA	908 radiographs (minimum disease duration of 3 years)	Modified LS	Not conclusive	0.5	Marinou et al.(42)
	rs1805010 (I50V)	600 RA	2846 radiographs (during 7 years)	SHS	NR	NS	Krabben et al. (41)
	rs1801275 (Q551R)	965 RA	912 radiographs (minimum disease duration of 3 years)	Modified LS	Protective	0.5	Marinou et al.(42)
	rs1801275 (Q551R)	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.01	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	NR	0.21 (meta-analysis)	
	rs4787423	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.03	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	NR	0.81 (meta-analysis)	

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
	rs1805015	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.04	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	NR	0.08 (meta-analysis)	
	rs7191188	600 RA	2846 radiographs (during 7 years)	SHS	Destructive	<0.01	Krabben et al. (41)
		1953 RA (meta-analysis of 6 cohorts)	3415 radiographs (3 repeated and 3 single measurement)	SHS and LS	NR	0.83 (meta-analysis)	
	rs6498016	600 RA	2846 radiographs (during 7 years)	SHS	Destructive	0.01	Krabben et al. (41)
		1953 RA (meta-analysis of 6 cohorts)	3415 radiographs (3 repeated and 3 single measurement)	SHS and LS	NR	0.88 (meta-analysis)	
	rs1805011	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.01	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	Four destructive	0.02 (meta-analysis)	
	rs1119132	600 RA	2846 radiographs (during 7 years)	SHS	Destructive	0.04	Krabben et al. (41)
		1953 RA (meta-analysis of 6 cohorts)	3415 radiographs (3 repeated and 3 single measurement)	SHS and LS	Three destructive and three protective	0.001 (meta-analysis)	

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL6	rs1800795 (–174)	930 RA	930 radiographs# (at baseline)	Modified LS	Protective	0.005	Marinou et al.(23)
	rs1800795 (–174)	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.64	Not published data.
	rs1524106	1418 RA (meta-analysis 4 cohorts: Leiden-EAC, Groningen, Lund, Sheffield)	4890 radiographs (3 repeated and 1 single measurement)	SHS and LS	Two protective and two destructive	0.006 (meta-analysis)	Not published data.
	rs7796691	1418 RA (meta-analysis 4 cohorts: Leiden-EAC, Groningen, Lund, Sheffield)	4890 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and three destructive	<0.001 (meta-analysis)	Not published data.
	rs7776857	1418 RA (meta-analysis 4 cohorts: Leiden-EAC, Groningen, Lund, Sheffield)	4890 radiographs (3 repeated and 1 single measurement)	SHS and LS	Two protective and two destructive	0.191 (meta-analysis)	Not published data.

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL10	rs1800896 (–1082)	108 early RA	At 2 years of follow-up*	Erosion vs no erosion	NR	NS	Cantagrel et al.(44)
	rs1800896 (–1082)	91 RA	At baseline, 3, 6 and 12 years*	SHS	Destructive (G) [†]	<0.02	Huizinga et al.(43)
	rs1800896 (–1082)	860 RA	860 radiographs# (at baseline)	Modified LS	Protective (A) [†]	0.01	Marinou et al.(23)
	rs1800896 (–1082)	600 RA (Leiden-EAC)	2846 radiographs (during 7 years)	SHS	Protective (A) [†]	0.92	Not published data.
		101 RA (Wichita)	337 radiographs (during 15 years)	SHS	Protective	0.122	
		649 RA (NDB)	649 radiographs	SHS	Protective	0.84	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Protective	0.09	
	rs1800872 (–592)	928 RA	928 radiographs# (at baseline)	Modified LS	Protective	0.006	Marinou et al.(23)
	Haplotype: –1082, –819, –592	95 RA	At baseline*	Erosive vs. non erosive	NA	NS	Pawlik et al.(45)

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL15	rs2322182	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	NS (meta-analysis)	Knevel et al.(46)
	rs7667746	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	4.8*10^-5 (meta-analysis)	Knevel et al.(46)
	rs7665842	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	3.6*10^-4 (meta-analysis)	Knevel et al.(46)
	rs4371699	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	0.01 (meta-analysis)	Knevel et al.(46)
	rs6821171	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Three protective and one destructive	0.01 (meta-analysis)	Knevel et al.(46)
TGFb	–869	173 RA	173 radiographs (at baseline)	LS	Protective	NS	Mattey et al.(51)
	–869	122 RA	122 radiographs	Modified Sharp score	Destructive	NS	Kim et al.(50)
FCRL3	rs7528684	117 RA	234 radiographs (at baseline and 10 years)	SHS progression	Destructive	0.02	Maehlen et al.(48)
	rs7528684	227 RA	227 radiographs (mean disease duration 6.3)	Modified Sharp score	Destructive	0.003	Han et al.(47)
	rs7528684	600 RA (Leiden-EAC)	2846 radiographs (during 7 years)	SHS plotted against disease duration	Destructive	0.57	Not published data.

w[∞]The direction of the effect size is presented, irrespective of the p-value of the obtained result. Hence, the direction was presented also of non-significant findings.
*Number of radiographs not reported in manuscript.
[#]These data were not reported in the manuscript, but derived from the data presented in the text or tables.
[†]The minor alleles were different between the cohorts. The allele with the lowest frequency in the specific cohort appears in parentheses.
RA, rheumatoid arthritis; ACPA, anti-citrullinated protein antibodies; SHS, Sharp van der Heijde score; LS, Larsen score; NR, not reported; NS, not significant; IL1B, interleukin 1 beta; IL4, interleukin 4; IL4R, interleukin 4 receptor; IL6, interleukin 6; IL10, interleukin 10; IL15, interleukin 15; TGFb, transforming growth factor beta; FCRL3, FC receptor-like protein 3; Leiden-EAC, Leiden early arthritis clinic; NARAC, North American Rheumatoid Arthritis Consortium; NDB, National Data Bank (American cohort).

IL4 and IL4R

SNPs in *IL4* and *IL4R* have been studied in several RA populations. IL4 is considered relevant, as it mainly promotes differentiation of T cells towards T_H2 cells. The role of IL4 is underscored by observations that IL4 knockout mice are characterized by extensive joint damage and that low synovial concentrations of IL4 have been detected in patients with established RA. Nevertheless, no clear associations have been detected between genetic variants in *IL4* and severity or progression of joint damage. The effect of IL4 is mainly mediated via the IL4Rα chain. An amino acid–changing variant in *IL4R* (rs1805010) was shown to associate with the presence of erosions.[40] Some indications were also found for functional readouts in this variant. Nonetheless, subsequent studies in UK and Dutch RA populations could not replicate the association between rs1805010 and progression of joint damage.[41,42] A recent study of tagging SNPs in *IL4R* observed and independently replicated 2 additional *IL4R* variants associated with radiographic progression (rs1119132 and rs1805011).[41] These 2 variants were in low linkage disequilibrium ($R^2 < 0.01$) with rs1805010. No data are available on the functional level of these 2 variants.

IL6

Marinou et al. found that rs1800795 in *IL6* was associated with less severe joint damage.[23] This association was not replicated in a Dutch cohort (Table 3), in which 3 other variants were associated with the severity of joint damage and were subsequently studied in other cohorts; 2 of these variants were significantly associated with joint damage in a meta-analysis that included the Dutch cohort (Table 3). Without the Dutch cohort, the association was no longer significant. Based on this observation and the difference in effect direction between the cohorts, no clear conclusions can be drawn on genetic variants in *IL6* and progression of joint damage.

IL10

Several lines of research suggest an important role for IL10 in the pathogenesis of joint destruction in RA. Preclinical studies showed that IL10 inhibits the generation of proinflammatory cytokines and the proliferation of T_H1 cells. In a rodent model of chronic arthritis, IL10 inhibited the severity of the disease.[43] The minor allele of a promoter polymorphism at position –1082 (rs1800896) was associated with less severe joint damage in a cohort of 91 female RA patients.[43] A similar significant observation was made in a UK study.[23] Furthermore, not significant findings were observed with a similar effect direction in several cohorts (Table 3).[44] Therefore, it would be very interesting to perform a meta-analysis of this variant, especially given the correlation observed between rs1800896 and expression, since patients with the severity risk allele produced lower IL10 levels. Similar findings were obtained by studying haplotypes of the –1082A/G, –819C/T, and –592A/C variants.[45] The –592 (rs1800872) variant in this haplotype ($R^2 = 0.29$ with rs1800896) was

also found to be associated with the severity of joint damage in one study, although other studies did not support this finding.[23;45] Whole-genome association studies showed that variants in the *IL10* gene were a relevant risk factor for Crohn disease and Behçet disease.

IL15

IL15 levels are increased in the serum, synovium, and bone marrow of patients with RA. IL15 influences both the innate and the adaptive immune response; it is mainly responsible for activation and proliferation of T cells. Emerging data show that this cytokine affects osteoclastogenesis. A tagging approach revealed several variants in *IL15* to be associated with the severity of progression of joint damage. Furthermore, in a meta-analysis of 4 cohorts, 4 variants were associated with progression.[46]

FCRL3

The Fc receptor–like family has potential immunoregulatory functions. FCRL3 has attracted research interest, since it is associated with several autoimmune diseases. It has been observed to be a risk factor for development of RA in Asian patients. It is preferentially expressed in B cells, and the –169C allele of *FCRL3* (rs7528684) has been associated with higher ACPA levels. In a Korean study, RA patients with the CC genotype have higher radiographic progression rates.[47] In a Norwegian study, ACPA-positive patients carrying both CC alleles also had more severe progression.[48] In a large Dutch cohort, no association was observed between *FCRL3* genotypes and radiographic progression in RA or ACPA-positive RA (Table 3). A meta-analysis of these data would be required to determine whether or not *FCLR3* is associated with severity of RA.

TGFβ1

The *TGFβ1* –869T/C variant was observed to be a susceptibility factor for RA in the Japanese population.[49] Subsequently, the *TGFβ1* –869T/C variant, which is related to the severity of joint damage, was investigated in 2 studies. In a Korean study, no significant association was observed.[50] A study from the UK found an association between this variant and joint damage, although the association was no longer significant when disease duration at the point the radiographs were taken was included in the analysis.[51] Considering the available data, this variant is not evidently associated with severity of joint damage.

Markers related to bone or cartilage

Destruction of bone or cartilage and the ability of bone and cartilage to resist inflammatory pressure may in part be explained by the patient's genetic constitution. Several markers have been studied to this end (Table 4).

RANK, RANKL, OPG, and TRAF6

The balance between osteoblast and osteoclast activity is crucial for healthy bone, and osteoclast-related bone loss is mediated by the OPG/RANK/RANKL/TRAF6 pathway. Receptor activator for nuclear factor κβ ligand (RANKL) is expressed and released by osteoblasts and activated T lymphocytes. RANKL promotes osteoclast formation and perpetuates its function and survival through binding of receptor activator of nuclear factor κβ (RANK). Subsequently, the RANK signal is mediated by TRAF6, a member of the TNF receptor associated factor (TRAF) protein family, which functions as a signal transducer in the NF-κβ family. The process of osteoclast formation and bone resorption is negatively regulated by osteoprotegerin (OPG), as binding of OPG inhibits activation of RANKL. The bone loss in RA points to an imbalance in the OPG-RANKL axis favoring bone resorption and resulting in erosion. This potential imbalance is supported by the association between the OPG/RANKL ratio in serum and joint destruction in RA. Genetic variants in *OPG*, *RANK*, and *RANKL* have been associated with bone mineral density and osteoporosis, and *TRAF6* has also been identified as a risk factor for development of RA. The variants tagging these 4 genes were evaluated recently in a candidate gene study. Variants that were significantly associated in the first cohort were subsequently studied in 3 additional cohorts. None of the variants in *RANK*, *RANKL*, or *TRAF6* were replicated after correction for multiple testing, although 1 variant in *OPG*, rs1485305, was significantly associated with more severe joint damage in a meta-analysis of the 4 cohorts and again after Bonferroni correction. This variant has also been associated with loss of bone mineral density (unpublished data) (Table 4).

DKK1, SOST, LRP5, and KREMEN1

Another pathway that is relevant for bone homeostasis is the canonical WNT/ß-catenin pathway, which involves binding of WNT proteins to a co-receptor complex comprising LRP5 or LRP6 and a member of the frizzled family of proteins. This binding leads to a signalling cascade resulting in the release of catenin in the cytoplasm, which eventually stimulates osteoblast differentiation. The cascade is negatively regulated by dickkopf 1 (DKK1) and sclerostin (SOST). DKK1 can also bind to cell surface receptor KREMEN1 and LRP5, thus strengthening the negative regulatory effect. Genetic variants in *LRP5* and *KREMEN1* were explored but not associated with the severity of progression of joint damage. However, several variants in *DKK1* and *SOST* were associated with progression of structural damage. In particular, when the severity risk alleles of both variants were present, a gene-gene interaction was observed, and patients with 4 risk alleles had very severe progression of damage. The relevance of the *DKK1* variants was substantiated by the finding that the risk genotypes were associated with higher serum DKK1 levels and that higher serum levels were associated with more severe joint damage in other studies. These data support the relevance of DKK1 for progression of joint damage (Table 4).[52]

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
RANK	rs17719830	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.04 (meta-analysis)	Knevel et al. *Submitted*
	rs17069845	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.09 (meta-analysis)	Knevel et al. *Submitted*
	rs11665260	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.10 (meta-analysis)	Knevel et al. *Submitted*
	rs12970081	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.08 (meta-analysis)	Knevel et al. *Submitted*
	rs8092336	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.003 (meta-analysis)	Knevel et al. *Submitted*
	rs6567279	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.02 (meta-analysis)	Knevel et al. *Submitted*
	rs17666267	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.01 (meta-analysis)	Knevel et al. *Submitted*
	rs8097062	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.22 (meta-analysis)	Knevel et al. *Submitted*
RANKL	rs931273	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.05 (meta-analysis)	Knevel et al. *Submitted*

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity (continued)

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
OPG	rs1353171	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.04 (meta-analysis)	Knevel et al. *Submitted*
	rs2326045	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.15 (meta-analysis)	Knevel et al. *Submitted*
	rs10955911	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.27 (meta-analysis)	Knevel et al. *Submitted*
	rs3102724	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	Knevel et al. *Submitted*
	rs2073618	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.001 (meta-analysis)	Knevel et al. *Submitted*
	rs1564861	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.01 (meta-analysis)	Knevel et al. *Submitted*
	rs1825511	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	Knevel et al. *Submitted*
	rs1485305	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	0.0002 (meta-analysis)	Knevel et al. *Submitted*
	rs1905785	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.41 (meta-analysis)	Knevel et al. *Submitted*
	rs1905776	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.17 (meta-analysis)	Knevel et al. *Submitted*
	rs6993813	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.59 (meta-analysis)	Knevel et al. *Submitted*

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity (continued)

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
DKK1	rs1896368	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and 3 destructive	0.001 (meta-analysis)	De Rooy et al.(52)
	rs10762715	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	De Rooy et al.(52)
	rs1528873	1022 RA (meta-analysis of 3 cohorts)	4494 radiographs (3 repeated)	SHS and LS	Three destructive	0.009 (meta-analysis)	De Rooy et al.(52)
	rs1441124	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	De Rooy et al.(52)
	rs1896367	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four protective	0.003 (meta-analysis)	De Rooy et al.(52)
	rs11001702	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.84 (meta-analysis)	De Rooy et al.(52)
	rs1194750	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	De Rooy et al.(52)
SOST	rs4792909	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Three protective and 1 destructive	0.01 (meta-analysis)	De Rooy et al.(52)
	rs6503475	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Two protective and 2 destructive	0.03 (meta-analysis)	De Rooy et al.(52)
	rs12600549	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.07 (meta-analysis)	De Rooy et al.(52)
KREMEN1	rs1322774	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.05 (meta-analysis)	De Rooy et al.(52)
LRP5	rs3736228	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.06 (meta-analysis)	De Rooy et al.(52)

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity (continued)

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
GRZB	rs8192916	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and 3 destructive	7.8*10^-4	Knevel et al.(53)
ADAMTS5	rs9984329	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and 3 destructive	0.46	Not published data.
	rs233601	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Three protective and 1 destructive	0.75	Not published data.
MMP3	5A/6A	96 early RA	192 radiographs (at baseline and 4 years)	SHS	Protective (5A)[∞]	0.04	Constantin et al.(57)
	5A/6A	308 RA	839 radiographs# from three time points	Ratingen score (modification of LS)	Destructive	NS	Dörr et al.(59)
	5A/6A	254 RA	254 radiographs	LS	Destructive (6A) [∞]	0.04	Mattey et al.(58)
MMP9	rs11908352	600 RA (Leiden-EAC)	3143 radiographs (during 7 years)	SHS	Destructive	1.75*10^-7	De Rooy et al. (60)
	rs11908352	686 RA (Wichita and NDB	926 radiographs (1 repeated and 1 single measurements)	SHS	Destructive	0.002	De Rooy et al. (60)

[∞]The direction of the effect size is presented, irrespective of the p-value of the obtained result. Hence, the direction was presented also of non-significant findings.
*Number of radiographs not reported in manuscript.
#These data were not reported in the manuscript, but derived from the data presented in the text or tables.
†The minor alleles were different between the cohorts. The allele with the lowest frequency in the specific cohort appears in parentheses.
RA, rheumatoid arthritis; SHS, Sharp van der Heijde score; LS, Larsen score; NR, not reported; NS, not significant; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator for nuclear factor κB ligand; OPG, osteoprotegerin; DKK1, dickkopf-related protein 1; SOST, sclerostin; KREMEN1, kringle containing transmembrane protein 1; LRP5, low density lipoprotein receptor-related protein 5; GRZB, granzyme B; ADAMTS5, a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 5; MMP3, matrix metallopeptidase 3; MMP9, matrix metallopeptidase 9.

GRZB

Granzyme B (GRZB) is a serine protease found in lytic granules of NK cells and cytotoxic T lymphocytes.[53] In vitro studies showed that granzyme B has enzymatic activity for the cleavage of aggrecan proteoglycans from cultured cartilage matrix.[53] The observations that loss of cartilage proteoglycans is an early event in the course of destructive arthritis and that many granzyme B–positive cells are present in the pannus of patients with RA increased interest in GRZB as a biomarker for progression of joint damage. Four cohorts were studied, and 1 polymorphism (rs8192916) was shown to increase the risk of a more destructive course of RA. Furthermore, mapping expression of quantitative trait loci in whole blood revealed that the risk alleles were also associated with higher levels of mRNA expression.[53]

ADAMTS5

ADAMTS5, previously known as aggrecanase 2, is a member of the large ADAMTS family of zinc-dependent proteases. Aggrecan is a major proteoglycan that is responsible for the compressibility and stiffness of cartilage.[54] One of the earliest changes observed in arthritis is depletion of cartilage aggrecan due to increased proteolytic cleavage within the interglobular domain.[55] Two major cleavage sites have been identified, and ADAMTS5 is thought to cleave aggrecan at one of these sites. Murine osteoarthritis models and inflammatory arthritis models supported the relevance of ADAMTS5 for aggrecan degradation.[56] Based on the hypothesis that variants in *ADAMTS5* might influence the severity of progression of joint damage, a candidate gene study was performed in 4 cohorts that were scored according to the Sharp van der Heijde method (in which the joint space narrowing score reflects the severity of loss of cartilage). No clear associations were observed in the 1418 patients and 4885 radiographs studied (Table 4).

MMP3

Matrix metalloproteinases (MMP) comprise a group of zinc- and calcium-dependent enzymes that are implicated in the destruction of articular cartilage and bone. MMP3 is abundantly present in the synovium and synovial fluid of RA patients and is considered to be the main MMP in cartilage degradation. It is secreted by fibroblasts, synovial cells, and chondrocytes and activates other MMPs, such as MMP9 and MMP2. Serum levels of MMP3 are elevated in both early and advanced RA, and elevated serum levels are correlated with more severe joint damage. Two studies reported a significant association between the promoter polymorphism *5A/6A* and joint destruction in patients with RA.[57,58] A third study did not find this association for *MMP3 5A/6A* itself, but identified a haplotype in this region that predisposed to more severe joint destruction.[59] Other authors reported an association between the *5A/6A* polymorphism and MMP3 serum levels and found that this promoter polymorphism was functionally relevant. Altogether, these data indicate an association

between this variant and the severity of joint damage in RA. Unfortunately, this *5A/6A* variant is not included in regular high-throughput platforms, and no data are available on the association between this variant and severity of joint damage in larger cohorts.

MMP9

Fewer data are available on the association between MMP9 and progression of RA. However, a genetic variant in *MMP9* has recently been identified as a risk factor for progression of joint damage (rs11908352). The variant was not identified using a candidate gene approach, but by evaluating polymorphisms located on 186 loci that were associated with autoimmune disorders and included on the Immunochip. rs11908352 was inserted into this platform because it is located near *CD40*. Nevertheless, it was observed to be associated with progression of joint damage, independently of variants in *CD40*. Furthermore, the risk allele for severity was also associated with higher serum MMP9 levels at disease onset. These data support the relevance of MMP9 for progression of joint damage in RA.[60]

Genome-wide study of progression of joint damage

In addition to the candidate gene studies described above, one genome-wide study investigated the ACPA-positive subset of RA. The study was performed in 3 stages. In the first stage, a cluster of SNPs located at chromosome 2q34 was found to be associated with progression of joint damage. In the second and third stages, rs7607479 was replicated as a risk factor for progression. The effect was protective, as patients carrying the minor allele had less severe joint damage. This polymorphism is located within the gene coding for sperm-associated antigen 16 (*SPAG16*), which is expressed in the joint and, more specifically, in fibroblast-like synoviocytes. Fibroblast-like synoviocytes carrying the minor allele expressed and secreted less MMP3, and RA patients with the minor allele had lower MMP3 levels in their serum. Subsequently, serum levels of MMP3 were associated with the severity of joint damage. These data point to a novel factor mediating MMP3 production and progression of joint damage and were further supported by the results of a multivariate analysis showing that when both the genetic variant and serum levels were included in the model, only serum levels were independently associated with progression of joint damage. Consequently, the effect of rs7607479 on joint damage is mediated via an effect on MMP3 production.[61]

Genetic factors: Conclusion

Many studies have evaluated genetic factors as risk factors or biomarkers for progression of joint destruction in RA. Given that genetic markers generally have small effect sizes in complex disorders, the number of patients and radiographs included in many of these studies was relatively low; consequently, the power of several studies was also low. Therefore, meta-analyses combining data from all available cohorts are necessary in order

to draw robust conclusions. For several of the markers discussed here, such meta-analyses are difficult with published data, since the outcome measures used are different. The genetic variants for which it would be particularly interesting to combine the data available in meta-analyses are rs2900180 in *C5/TRAF1*, rs1800896 in *IL10*, rs1800795 in *IL6*, and rs758684 in *FCLR3*. Moreover, current data suggest that the *5A/6A* variant in *MMP3* is also associated with joint damage. Further validation in larger studies would be useful.

Nonetheless, meta-analyses have been performed and published, and in all of the cohorts included, the severity of progression of joint damage was measured quantitatively (Sharp van der Heijde and Larsen scores). Variants in 10 genes have been significantly associated with progression of joint damage in several independent studies and in meta-analyses (Figure 2). These variants are related to inflammation (eg, *IL2RA*, *IL4R*, and *IL15*), autoimmunity (eg, *HLA-DRB1*), bone homeostasis (eg, *DKK1*, *SOST*, *OPG*), and cartilage destruction (eg, *GRZB*). Some of these genetic variants were also associated with different expression levels in blood serum and blood plasma (eg, *IL2RA, DKK1, MMP9, GRZB*).

SEROLOGIC BIOMARKERS OF RADIOGRAPHIC JOINT DESTRUCTION IN RA

Serologic markers associated with disease severity include autoantibodies, other markers related to inflammation and proteinases, and components of the extracellular matrix of bone and cartilage (Table 5).

Autoantibodies

Many studies have shown that both rheumatoid factor and ACPA are independently associated with a destructive disease course.[62-70] The most widely studied ACPA are the anti-CCP2 antibodies. Van der Linden et al compared the anti-CCP2 test with the anti-CCP3 test and the anti-MCV test for predicting radiographic progression in early RA patients. When each test was performed alone, all 3 had comparable predictive abilities. Furthermore, when the results of the anti-CCP2 test were known, the other tests had no additive value, indicating that a single ACPA test is sufficient for estimation of risk in RA.[71]

Other autoantibodies have also been explored in RA. One such autoantibody acts directly against the protein arginine deaminase type 4 (PAD4). PAD are enzymes that catalyze the citrullination reaction. PAD4 has been detected in inflamed synovium, and anti-PAD4 antibodies were associated with joint damage and erosions in a single cross-sectional study.[72] However, no confirmative studies have been reported since. Novel autoantibodies also include the anti-Carp antibodies (anti-carbamylated protein antibodies). Carbamylation is also a post-translational modification. This reaction is enhanced in smokers, in patients with renal failure, and in (chronic) inflammation. Anti-Carp antibodies are prevalent in RA. In ACPA-negative patients they were associated with the severity of joint destruction in

Table 5 Serological Markers of Bone and Cartilage Damage.

Marker	Patients	Radiographs	Outcome	Effect elevated marker level at baseline	P Value	Reference
MMP3	116 early RA	232 radiographs# (at baseline and 1 year)	Progression in modified Sharp score	Destructive	<0.01	Garnero et al.(76)
	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	<0.0001	Young-Min et al.(79)
	36 RA	72 radiographs (at baseline and 2 years)	Progression in SHS	NR	NS	Den Broeder et al.(80)
	109 RA	At baseline, 1 and 2 years*	Progression in SHS	Destructive	0.001	Tchetverikov et al.(75)
	98 early RA	196 radiographs# (at baseline and 1 year)	Progression in LS	Destructive	<0.05	Green et al.(116)
	26 early RA	78 radiographs (at baseline, 6 months, 12 months)	Progression in LS	Destructive	<0.05	Yamanaka et al.(117)
	32 RA	At baseline, after the first 6 months (with radiographic progression) and after the second 6 months (with no radiographic progression)*	SHS radiographic progression (≥ 5 points) vs no radiographic progression (≤ 1 point)	Destructive	<0.05	Posthumus et al.(118)
	24 RA	At baseline and yearly during 5 years*	LS: 3 groups: slow, intermediated and rapid progression	Destructive	<0.01	Roux-Lombard et al.(81)
	46 RA	At baseline and every 6 months to 2 years*	LS: erosive vs non-erosive	Destructive	0.02	Jensen et al.(83)
CTX-I (b-C-telopeptide)	279 early oligo- and polyarthritis	At baseline and after 2 years*	Progression ≥5 SHS	Destructive	<0.001	Jansen et al.(77)
	190 RA	380 radiographs (at baseline and 2 years)	Erosion vs no erosion	Destructive	0.15	Le Loët et al.(78)
	110 RA	At baseline, week 28, week 56 and at end of study (median of 4 years)*	Modified SHS: annual progression rate	Destructive	0.01	Garnero et al.(82)

Table 5 Serological Markers of Bone and Cartilage Damage. (continued)

Marker	Patients	Radiographs	Outcome	Effect elevated marker level at baseline	P Value	Reference
CTX-II	110 RA	at baseline, week 28, week 56 and at end of study (median of 4 years)*	Modified SHS: annual progression rate	Destructive	0.02	Garnero et al.(82)
	116 early RA	232 radiographs# (at baseline and 1 year)	Progression in modified Sharp score	Destructive	<0.01	Garnero et al.(82)
	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.003	Young-Min et al.(79)
COMP	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.004	Young-Min et al.(79)
	36 RA	72 radiographs (at baseline, 1 and 2 years)	Progressor vs nonprogressor	Destructive	0.01	Den Broeder et al.(80)
	24 RA	At baseline and yearly during 5 years*	LS: 3 groups: slow, intermediated and rapid progression	Not conclusive	NS	Roux-Lombard et al.(81)
	183 early RA	283 radiographs (at 5 and 10 years)	LS	Destructive	<0.05 (5 years) >0.05 (10 years)	Lindqvist et al.(67)
TIMP1	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.02	Young-Min et al.(79)
	24 RA	At baseline and yearly during 5 years*	LS: 3 groups: slow, intermediated and rapid progression	Not conclusive	NS	Roux-Lombard et al.(81)

Table 5 Serological Markers of Bone and Cartilage Damage. (continued)

Marker	Patients	Radiographs	Outcome	Effect elevated marker level at baseline	P Value	Reference
PYD	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.04	Young-Min et al.(79)
	190 RA	380 radiographs (at baseline and 2 years)	Erosion vs no erosion	Destructive	0.0004	Le Loët et al.(78)
	46 RA	At baseline and every 6 months to 2 years*	LS: erosive vs non-erosive	Destructive	0.07	Jensen et al.(83)
	437 RA	At baseline and yearly during 7 years*	SHS	Destructive	0.0001	Krabben et al. *in press*(84)
Glc-Gal-PYD	116 early RA	232 radiographs# (at baseline and 1 year)	Progression in modified Sharp score	Destructive	<0.01	Garnero et al.(76)
	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.04	Young-Min et al.(79)
OPG / RANKL ratio	92 RA	at baseline and yearly during 5 years*	Modified SHS	Protective (OPG: RANKL)	0.001	Geusens et al.(86)
	44 RA	60 radiographs#	LS	destructive (OPG) protective (RANKL)	0.01 (OPG) 0.85 (RANKL)	Skoumal et al.(85)
	100 early RA	Mean of eight radiographs per patient (max follow-up of 11 years)*	SHS: annual progression rate	Destructive (RANKL:OPG)	<0.001	Van Tuyl et al.(87)
CXCL13	74 RA (BeSt)	At 4 years diease duration	SHS	Destructive	0.02	Meeuwisse et al.(88)
	155 RA (EAC)	At baseline and yearly during 7 years*	SHS	Destructive	<0.001	Meeuwisse et al.(88)

°°The direction of the effect size is presented, irrespective of the p-value of the obtained result. Hence, the direction was presented also of non-significant findings.
*Number of radiographs not reported in manuscript.
#These data were not reported in the manuscript, but derived from the data presented in the text or tables.
RA, rheumatoid arthritis; SHS, Sharp van der Heijde score; LS, Larsen score; NR, not reported; NS, not significant.
MMP3, matrix metallopeptidase 3; CTX-I, carboxy-terminal collagen crosslink I; CTX-II, carboxy-terminal collagen crosslink II; COMP, cartilage oligomeric matrix protein; TIMP1, tissue inhibitor of metalloproteinases 1; PYD, pyridinoline; Glc-Gal-PYD, glycosylated pyridinoline; OPG/ RANKL ratio; osteoprotegerin/ receptor activator of NF kB ligand ratio; CXCL13, chemokine (C-X-C motif) ligand 13;
BeSt, 'behandel strategieën voor rheumatoïde arthritis'; EAC, early arthritis clinic.

an early RA population.[73] Since this association has thus far not been replicated, the value of these novel antibodies as biomarkers for progression of joint damage in RA remains undetermined.

Acute-phase reactants

Since RA is an inflammatory disease, it is no surprise that C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) are associated with the severity of disease course. Nonetheless, these markers explain only a fraction of the total variance in joint destruction. A recent study calculated the variance of joint destruction explained by cumulative inflammation (area under the curve of serial CRP levels over time) to be 15-19%.[74] Hence, other markers or processes also play a role.

MMP3

The function of MMP3 has been discussed above. There is overwhelming evidence that serum MMP3 levels are associated with progression of joint damage, as a positive association has been reported in 8 of the 9 studies on this subject. The only study that did not reveal an association was small and analyzed only 36 patients. Intriguingly, several of the other studies that reported higher serum levels to be associated with future joint damage were small (24-46 patients). Given that hundreds of patients were necessary to identify genetic variants, it is clear that the effect size of serum markers is generally larger than that of genetic markers. It is noteworthy that MMP3 levels are increased throughout disease course, thus making it a stable biomarker of progression.[75]

CTX-I and CTX-II

Urinary C-terminal crosslinking telopeptide type I (CTX-I) and type II (CTX-II) collagen are markers of bone and cartilage degradation. CTX-I was associated with the severity of joint damage in all the studies that measured this parameter.[76,77] Only one study could not detect this association, although it evaluated the presence but not the severity of erosive disease.[78] Interestingly, CTX-I was a potent predictor whose effect was independent of the association between progression of joint damage and rheumatoid factor, disease activity score, or ESR. CTX-II is a specific marker of type II collagen cleavage in cartilage. Excretion of CTX-II was predictive of future joint damage, independently of other inflammatory markers.[76,79] None of these studies made adjustments for ACPA status.

COMP

Cartilage oligomeric matrix protein (COMP) is expressed at high levels in the matrix of chondrocytes. This marker was increased in patients with a more destructive disease course. It is interesting to note that significant associations were established in relatively small studies. It has not been determined whether or not the association between COMP

and joint damage was independent of other biomarkers.[67,79-81] In the largest study to date (containing 183 RA-patients), serum COMP was an independent predictor for joint damage after 5 years, although after 10 years of disease, this association was lost and only anti-CCP and CRP were independently associated with the severity of damage in hand and foot joints.[67]

TIMP

It is unclear whether tissue inhibitor of metalloproteinases 1 (TIMP) is a biomarker for severe destructive RA. Two studies have been published, both with the quantitative Larsen score as the outcome; an association was observed in one study but not in the other.[79,81]

PYD

Pyridinoline (PYD) is a major cross-linking compound of collagen fibers in cartilage that is present in the collagen of bone and tissues such as synovium. Pyridinoline levels are higher in RA patients than in healthy persons and patients with other rheumatologic disorders. In addition, some cross-sectional studies indicated that pyridinoline levels are higher in cases of active or severe RA. Prospective studies have been performed based on serum and urine pyridinoline levels. Both markers were elevated in patients who developed more severe joint destruction.[78,79,82-84] Intriguingly, this serum marker was also predictive in the early and advanced stages of RA, suggesting that it is also a stable biomarker for severity of joint damage in RA.[84]

RANKL/OPG

The genetic variants in OPG and RANKL have been discussed above. OPG is a soluble decoy receptor produced by osteoblasts that inhibits differentiation of the osteoclast precursor by neutralizing the receptor activator of NF-κβ ligand (RANKL). Although it was first observed that serum OPG levels were associated with joint damage in RA,[85] a subsequent study by Geussens et al revealed that the RANKL/OPG ratio in particular is predictive of joint destruction.[86,87]

CXCL13

CXCL13 is also known as B lymphocyte chemo-attractant and has been reported to interact with the receptor CXCR5, which is expressed by B cells and follicular B helper T cells. High levels of CXCR5 were also found in human osteoblasts, and activation by its ligand CXCL13 induced the release of extracellular matrix–degrading enzymes. CXCL13 levels are elevated in the serum of patients with RA. Based on these observations, CXCL13 could play an important role in the process of bone remodelling. Indeed, high CXCL13 levels were shown to be associated with more severe joint destruction over time in 2 Dutch

cohorts. This biomarker was most valuable in the anti–CCP-2–negative subpopulation of RA patients.[88]

IL2RA

IL2RA (CD25), the high affinity α chain of the IL2 receptor, is expressed on many immune cells and measurable in serum after cleavage from the membrane. It is considered a marker of T-cell proliferation. Several studies in other autoimmune diseases and healthy persons showed that the genetic variant described above (rs2104286) is associated with higher serum levels. In RA, higher serum levels are associated with more severe joint damage.[34] If this association is replicated in other cohorts, it could prove to be a relevant biomarker that is also more easily measurable than the genetic variant.

IMAGING BIOMARKERS AND THE SEVERITY OF RADIOGRAPHIC JOINT DESTRUCTION IN RA

The most frequently investigated imaging biomarkers for predicting radiographic joint destruction in RA patients are markers that are visible with MRI and ultrasound, namely, bone marrow edema, synovitis, tenosynovitis, and erosions.

MRI

MRI is increasingly used to measure disease states and treatment response in RA research. MRI has important advantages over conventional radiographs; in particular, it makes it possible to visualize and quantify inflammation of synovium, tendons, and bone (bone marrow edema), as well as structural damage. Bone marrow edema is common in RA and is estimated to occur in 68%-75% of patients with early RA.[89] Bone marrow edema is not detected by ultrasound or other imaging modalities and is a strong predictor of erosive progression. In a randomized controlled trial consisting of 130 RA patients, Hetland et al showed that bone marrow edema is an independent predictor of 2-year radiographic progression (coefficient, 0.59-0.75; $P<0.001$; $R^2=25\%-41\%$). Bone marrow edema also predicted radiographic progression at 5 years (coefficient, 0.83; $P<0.001$; $R^2=23\%$).[90;91] In an observational cohort of 84 early RA patients, Boyesen and Haavardsholm et al identified baseline bone marrow edema as an independent predictor of both 1-year radiographic erosive progression (OR=2.8, $P=0.04$) and 1-year MRI erosive progression (OR=1.3; $P=0.04$).[92-94] In an observational cohort of 42 patients, McQueen et al also demonstrated that 1-year radiographic erosions were more frequent in patients who had a total MRI score at baseline > 13 (OR=12.4; $P=0.002$)[95]. Additionally, they followed 31 of these patients over 6 years and observed that bone marrow edema at baseline also predicted the severity of radiographic joint destruction after 6 years ($R^2=0.20$; $P=.01$).[96] In an observational cohort of 24 early RA

patients, Lindegaard et al showed an RR of 4.0 for 1-year radiographic erosion when bone marrow edema was observed on the MRI; when erosion was observed on the MRI scan, the RR was 12.1.[97] In an observational cohort of 40 early RA patients, Conaghan et al showed a clear relationship between baseline MRI synovitis and development of subsequent MRI erosive destruction (area under the curve for MRI synovitis, r=0.42, $P<0.007$).[98] However, they did not assess the relationship with radiographic destruction. Boyesen et al showed that baseline synovitis on MRI independently predicted 3-year radiographic progression (beta=0.14; $P=0.03$).[92] Altogether, these studies confirm that the presence of synovitis and bone marrow edema on MRI is predictive of radiographic progression in patients with early RA. Again, the number of patients included in these studies was relatively low, and the observed effect sizes large.

An association between inflammation markers on MRI and radiographic joint destruction cannot always be identified. This can in part be explained by the short follow-up; assuming that bone marrow edema is a pre-erosive lesion, it takes time for a lesion to evolve from osteitis to erosion. Hoving et al observed that only half of the patients with erosions on MRI at baseline progressed to erosions on radiographs after a follow-up of 6 months.[99] Kamishima et al did not find a significant correlation between bone marrow edema and 1-year radiographic progression in 29 RA patients treated with anti-interleukin 6 receptor antibody. However, they did find a significant correlation between erosion on MRI and 1-year radiographic progression.[100] Furthermore, Ostergaard et al showed that MRI is considerably more sensitive than conventional radiographs; most new radiographic erosions (78%) were visualized at least 1 year earlier by MRI than by conventional radiograph, and MRI detection of new radiographic erosions preceded radiographic detection by a median of 2 years.[101] In a sample of 16 RA patients, Scheel et al showed that 41% of the erosions on MRI at baseline were seen on the radiograph at 7 years.[102] Although not every bone edema lesion evolves to erosion on the radiograph, most studies show that the development of radiographic erosions in the short term was highly unlikely in the absence of baseline MRI inflammatory changes. McQueen et al found a positive predictive value of 0.53 and a negative predictive value of 0.92 for bone marrow edema at baseline and radiographic erosions at 1 year.[95] Mundwiler et al reported similar findings only in the metatarsophalangeal joints of RA patients.[103]

Ultrasound

Although MRI is a potentially powerful technique for evaluation of inflammation and structural damage in RA, it is not a routine procedure owing to limited availability and high costs. Ultrasound is more available, less expensive, and does not require administration of contrast medium. It can be used to evaluate inflammation of joints and tendons and erosions. Synovitis is usually scored semi-quantitatively for both gray scale synovitis and power Doppler activity.

In an observational cohort of 84 RA patients, Boyesen et al showed that ultrasound gray-scale inflammation predicted 1-year MRI erosive progression (OR=2.01, p=0.02).[92] In their study of 59 RA patients starting anti-TNF therapy, Dougados et al. showed that baseline synovitis increased the risk of structural radiographic progression. The results of this study also implied that ultrasonographic examinations are not superior to clinical examination for predicting structural radiographic progression in RA.[104]

Tenosynovitis of the extensor carpi ulnaris was an imaging biomarker with an independent predictor for MRI erosive progression in the cohort of Lillegraven et al.[105]

Early RA bone erosions are detected with greater sensitivity using ultrasound than using conventional radiography, most probably as a direct function of their size. Szkudlarek et al. found more and larger erosions in the finger and toe joints of patients with established RA than in early RA.[106] In their study of 16 RA patients, Scheel et al showed that 22% of the erosions on ultrasound at baseline were seen on radiograph at 7 years; this percentage was lower than that detected by MRI.[102]

Ultrasound is limited by its capacity to detect lesions at some locations, such as the wrist and intra-articular surfaces of the third and fourth metacarpophalangeal joints. In addition, discrimination between cortical irregularity and erosions is problematic. Furthermore, with ultrasound, it is not possible to visualize inflammation of the bone (bone marrow edema). Ultrasound is also highly operator-dependent and time-consuming.

More research is necessary before ultrasound can be considered a useful tool for predicting radiographic progression of joint damage in RA.

COMBINING BIOMARKERS

Treatment of RA is not adjusted to individual prognoses but to measured disease activity. Consequently, some RA patients may be undertreated if disease activity is not suitably suppressed (generally patients with severe disease). Undertreatment could be attributed to the fact that aggressive combination therapy or biologics are not universally prescribed because of costs and concerns over toxicity. This reasoning underlines the need for a good prediction metric to identify patients with a potentially severe disease course. Although several prediction models or risk matrices have been developed, none have been validated. Furthermore, these models adequately predicted outcome in only about 50% of patients. Better models are necessary.

The existing models for evaluation of the severity of the course of RA are based on CRP, ACPA titer, baseline erosions, and the number of swollen joints.[69,107-110] This review of biomarkers of progression in RA shows that most of the biomarkers identified had not yet been integrated in prediction models. The addition of more recently identified genetic, serologic, and imaging markers will increase predictive ability.

CONCLUSION

Progression of joint destruction is an objective measure of the severity of RA and is frequently used to identify biomarkers that can be used to evaluate disease course. The highest sensitivity is observed with hand and foot radiographs taken serially over time and scored using a validated quantitative scoring method. Precise measurements increase statistical power, which is very relevant in genetic studies, since most genetic factors have small effect sizes. Serologic and imaging factors generally have larger effect sizes, probably because they are more closely related to the phenotype (Figure 3). The present manuscript provides an overview of genetic markers of severity studied in multiple cohorts; several markers for which a positive association was established in older studies were not replicated in more recent studies. For some markers, no definite conclusion could be drawn, thus necessitating a meta-analysis. Nonetheless, more than 10 genetic risk factors have been identified and replicated. Furthermore, various serologic and imaging risk factors were described. Most of these known risk factors have not yet been included in risk models. Combination of these markers to achieve adequate predictive value requires further study.

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