7

A Genetic Variant in *Granzyme-B* Is Associated With Progression of Joint Destruction in Rheumatoid Arthritis

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ABSTRACT

Objective

Genetic factors account for an estimated 45–58% of the variance in joint destruction in rheumatoid arthritis (RA). The serine proteinase granzyme B induces target cell apoptosis, and several in vitro studies suggest that granzyme B is involved in apoptosis of chondrocytes. Serum levels of granzyme B are increased in RA and are also associated with radiographic erosions. The aim of this study was to investigate *GZMB* as a candidate gene accounting for the severity of joint destruction in RA.

Methods

A total of 1,418 patients with 4,885 radiograph sets of the hands and feet from 4 independent cohorts were studied. First, explorative analyses were performed in 600 RA patients in the Leiden Early Arthritis Clinic cohort. Fifteen single-nucleotide poly-morphisms (SNPs) tagging *GZMB* were tested. Significantly associated SNPs were genotyped in data sets representing patients from the Groningen, Sheffield, and Lund cohorts. In each data set, the relative increase in the annual rate of progression in the presence of a genotype was assessed. Data were summarized in a meta-analysis. The association of *GZMB* with the RNA expression level of the *GZMB* genomic region was tested by mapping expression quantitative trait loci (QTLs) on 1,469 whole blood samples.

Results

SNP rs8192916 was significantly associated with the rate of joint destruction in the first cohort and in the meta-analysis of all data sets. Patients homozygous for the minor allele of rs8192916 had a higher rate of joint destruction per year compared with other patients (P 7.8 x 10^{-4}). Expression QTL of *GZMB* identified higher expression in the presence of the minor allele of rs8192916 (P 2.27 x 10^{-5}).

Conclusion

SNP rs8192916 located in *GZMB* is associated with the progression of joint destruction in RA as well as with RNA expression in whole blood.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder that affects 0.5–1% of the population and is associated with significant morbidity, disability, and cost to society. Radiographic joint destruction reflects the cumulative burden of inflammation and is considered to be an objective measure of the severity of RA[1]. The degree of joint destruction varies significantly between patients. The processes behind this difference are incompletely understood. Inflammatory markers and auto-antibodies are potent risk factors for joint destruction but explain ~30% of the variance in joint destruction[2]. Results of a twin study suggested that genetic factors influence the severity of joint destruction in RA, and a recent study in the Icelandic RA population estimated the heritability rate of joint destruction to be ~45–58%.[3,4] Hence, to increase understanding of the processes mediating joint destruction, it would be beneficial to study genetic variants that could predispose to a severe outcome of RA.

Here, we describe a candidate gene study of the association of *GZMB* with the rate of joint destruction. Granzyme B is a serine protease found in the lytic granules of natural killer (NK) cells and cytotoxic T lymphocytes. When granzyme B is secreted into the interspace between the NK cell and the target cell, it triggers cell death by apoptosis.[5,6] In vitro studies have shown that granzyme B has enzymatic activity for the cleavage of aggrecan proteoglycans from cultured cartilage matrix and whole cartilage explants;[7,8] loss of cartilage proteoglycans is an early event in the course of destructive arthritis.[9] In addition, studies of cartilaginous tissue demonstrated the presence of many granzyme B–positive cells among the chondrocytes in the pannus lesion.[8] Furthermore, the levels of granzyme B were shown to be increased in the synovial fluid of patients with RA compared with that of healthy control subjects,[10] and increased serum levels were associated with early development of radiographic erosions.[11] Finally, a genetic variant (rs854350) in *GZMB* is reported to associate with susceptibility to RA.[12]

These data led us to hypothesize that genetic variants in *GZMB* are associated with the severity of joint destruction in RA. We tested this hypothesis using 4 data sets comprising data for European RA patients for whom longitudinal radiography data on joint destruction were available. All data sets included patients in whom the diagnosis was made during a period when treatment strategies were less aggressive and less controlled than they are currently. These conservative treatment strategies made the data sets suitable for the present study, because the natural disease course was less inhibited. To further investigate the findings, the single-nucleotide polymorphisms (SNPs) associated with progression of joint destruction were also tested for their association with RNA expression.

PATIENTS AND METHODS

Study population

Four data sets comprising data for adult European patients with RA were studied. RA was defined according to the 1987 American College of Rheumatology criteria[13] in all data sets except the Lund data set, for which the 1958 criteria for RA[14] were used. Radiographs of both the hands and feet were available for all patients (Table 1). All patients provided informed consent, and approval was obtained from the local ethics committee for each study.

Table 1: Characteristics for each dataset.

Cohort	Leiden-EAC (n=600)	Groningen (n=275)	Sheffield (n=396)	Lund (n=147)
Year of diagnosis	1993-2006	1945-2001	1938-2003	1985-1990
Follow-up years*	7 years	14 years	Not applicable*	5 years
Total no. of X-ray sets	2,846	862	396	781
Method of scoring	SHS	SHS	Larsen	Larsen
Female n (%)	412 (69)	194 (71)	290 (73)	98 (67)
Age at diagnosis, mean ± SD	56 ± 16	49 ± 13	46 ± 13	51 ± 12
Anti-CCP+ n (%)	323 (55)	160 (80)	302 (79)	114 (80)

SHS= Sharp-van der Heijde score

*Data of Leiden-EAC, Groningen and Lund were from baseline onwards during respectively 7, 14 and 5 years of follow-up. The data of Sheffield were collected once during the disease period, the mean disease duration was 15 years (range 3-65 years).

Leiden Early Arthritis Clinic (EAC) cohort

This data set represented 600 patients with early RA from the western part of The Netherlands, who were included in the Leiden EAC between 1993 and 2006.[2] Patients were included at the time of diagnosis and were followed up annually. Radiographs were obtained at baseline and at yearly follow-up visits for 7 years. A total of 2,846 sets of radiographs of the hands and feet were available. All radiographs were chronologically scored by one experienced reader who was unaware of the genetic or clinical data, using the Sharp/van der Heijde (SHS) scoring method for the hands and feet.[15] A total of 499 randomly selected radiographs were scored twice. The within-reader intra class correlation coefficient (ICC) was 0.91. Treatment of the patients could be divided into 3 time periods. Patients included in 1993–1995 were initially treated with non-steroidal anti-inflammatory drugs, patients included in 1996–1998 were initially treated with chloroquine or sulfasalazine, and patients included after 1999 were promptly treated with methotrexate or sulfasalazine.

Groningen cohort

The second set of data represented 275 RA patients from the northern part of The Netherlands, in whom RA was diagnosed between 1945 and 2001. The duration of follow-up after diagnosis was limited to 14 years. The mean number of radiograph sets (hands and feet) per patient was 3.1 (maximum of 8 radiographs per patient). The total number of radiograph sets was 862. The radiographs were scored chronologically by 1 of 2 readers, using the SHS method. The ICC within readers was >0.90 and between readers was 0.96. In this data set, patients in whom RA was diagnosed before 1990 had, on average, a more progressive course of joint destruction compared with patients in whom RA was diagnosed after 1990. This difference in joint destruction progression is consistent with the approach of initiating disease-modifying anti-rheumatic drug (DMARD) treatment early, which was introduced in the 1990s.

Sheffield cohort

The third data set represented 396 RA patients from the Sheffield, UK area. RA patients for whom radiographs were available were recruited from the Rheumatology Department of the Royal Hallamshire Hospital in Sheffield between 1999 and 2006.[16] RA patients were assessed once during their disease course. The mean ± SD disease duration at the time of the assessment was 15 ± 11 years (range 3–65 years). Radiographs of the hands and feet were scored by one reader using a modification of the Larsen score.[17] Ten percent of the radiographs were scored twice to quantify intra-observer variation, using a weighted kappa value of 0.83.[16]

Lund cohort

The fourth data set represented 183 Swedish patients with early RA who were prospectively followed up yearly over 5 years; radiographs and DNA were available for 147 of these patients.[18,19] Patients were recruited from primary care units in the Lund area from 1985 to 1989. Radiographs of the hands and feet were obtained at the start of the study and then annually for 5 years, resulting in a total of 781 sets of radiographs. Radiographs were scored chronologically by one of two readers, according to the Larsen method.[20] The ICC between readers, as determined based on 105 radiographs, was 0.94. During the inclusion period, immediate DMARD therapy was not common, and only half of the patients were receiving any DMARD at the 5-year follow-up. The DMARDs used most commonly were chloroquine, D-penicillamine, sodium aurothiomalate, and auranofin.[18]

SNP selection and genotyping

The region of *GZMB* plus the haplotype block upstream and downstream of the gene were tagged using the pairwise Tagger algorithm developed by de Bakker et al[21] and implemented in Haploview.[22] Two SNPs (rs8192917 and rs2236338) were known to be amino

acid–changing SNPs. One intergenic SNP, rs854350, had a significant association with RA susceptibility in the data set of the Wellcome Trust Case Control Consortium.[12] These 3 SNPs were force included. Pairwise-tagging SNPs were selected from the CEPH/CEU HapMap data set (phase II, release 21, NCBI build 35) using Haploview software (minor allele frequency [MAF] >0.05, pairwise $r^2 > 0.8$). Sixteen SNPs captured *GZMB*. Multiplex SNP arrays were designed using an Illumina GoldenGate platform, according to the protocols recommended by the manufacturer. One SNP could not be designed (rs1951601), but a good proxy (rs12433772; $r^2 = 0.90$) was typed instead. The final SNP selection and the linkage disequilibrium (LD) information are shown in Figure 1.

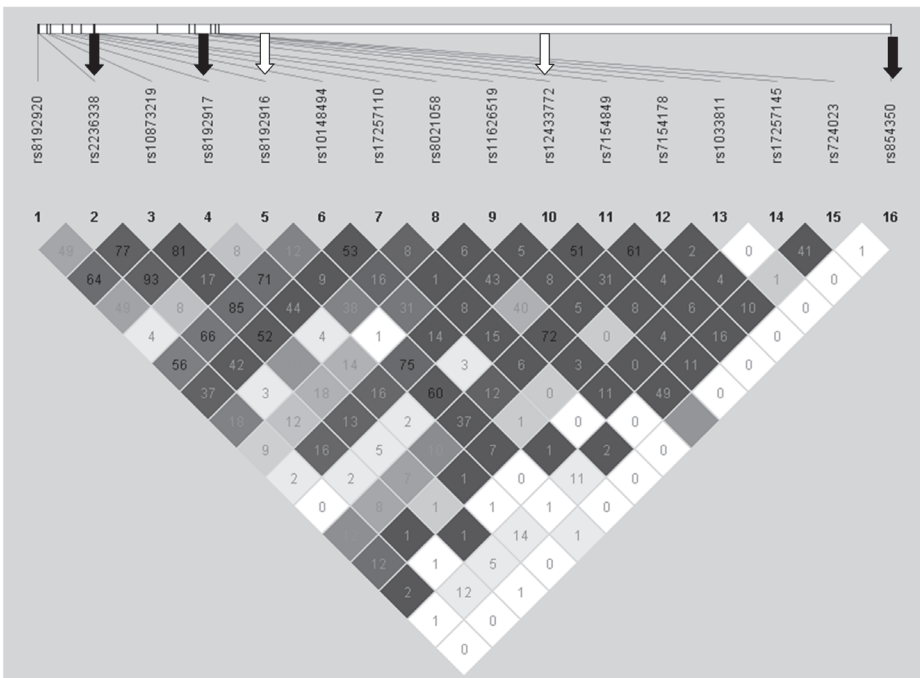

Figure 1 LD structure between of 16 tag-SNPs in GZMB.
The depicted data are from 600 Leiden RA-patients. The numbers present the r^2 between the SNPs. The colours refer to D´. Black arrows point out the amino acid changing SNPs, rs2236338 and rs8192917, and the susceptibility SNP of WTCCC, rs854350. These SNPs were forced to include. One SNP, rs1951601, could not be designed but a good proxy existed: rs1243372, r^2=0.90. One SNP, rs8192920, was not analyzed because of low success rate of typing. Significant SNPs in the analyses on the Leiden dataset are marked by a white arrow.

Software supplied by Illumina was used to automatically identify the genotypes. Each 96-well plate consisted of one positive and one negative control. Clusters were evaluated, and all doubtful calls were checked. After manually evaluating the spectra of each cluster, the genotypes were accepted, recalled, or rejected. At least 12% of the genotypes were

assessed in duplicate, with an error rate of <1% for all SNPs. One SNP, rs8192920, had a success rate of 75% and was therefore excluded from further analysis. The remaining SNPs were typed with a success rate of >95% (additional information is available from the corresponding author). None of the SNPs deviated from Hardy-Weinberg equilibrium ($P < 0.001$).

The SNP that was significantly associated with joint destruction in the first cohort was genotyped in the other 3 cohorts. The SNP was genotyped as a part of multiplex SNP arrays designed with Sequenom iPLEX, according to the protocols recommended by the manufacturer. Software supplied by the same manufacturer was used to automatically identify the genotypes. Each iPLEX consisted of at least 9 positive controls and 9 negative controls. All doubtful calls were checked manually; DNA samples in which >30% of the SNPs failed were excluded from analysis (n = 31). At least 5% of the genotypes were assessed in duplicate, with an error rate of <1%. The success rates were all >95%. None of the SNPs deviated from Hardy-Weinberg equilibrium.

Measuring and analyzing *GZMB* expression levels

The SNP that remained significant after meta-analysis was tested for its association with RNA expression of the genomic region of *GZMB*, by mapping expression quantitative trait locus (eQTL) on a data set representing peripheral blood samples obtained from 1,469 unrelated individuals.[23,24] RNA expression was tested using probes with a midpoint position <250 kb from the tested SNP, thus testing for a *cis* effect. Expression QTL effects were determined using Spearman's rank correlation coefficient. If an association was observed, it was determined whether other SNPs in the region (n = 583) had a stronger *cis*-eQTL effect. If this was the case, the first analysis was repeated using linear regression with adjustment for the strongest associating SNP, in an effort to determine the eQTL effect of the assessed SNPs independently of LD with the most strongly associated SNP. Genotypes were imputed with HapMap2 (release 24), using Impute version 2. Only SNPs with a MAF >5% and a Hardy-Weinberg *P* value greater than 0.0001 were included for analysis. Correction for multiple testing was performed by controlling the false discovery rate at 5%, permuting the gene expression labels 100 times as previously described.[25] Finally, to prevent false-positive findings due to primer polymorphisms, SNP–probe combinations were excluded from the *cis*-eQTL analysis when the 50-bp long expression probe mapped to a genomic location that contained a known SNP that was showing at least some LD ($r^2 > 0.1$) with the *cis* SNP.

Statistical analysis

Associations between genotypes and radiographic joint destruction were analyzed in 2 phases. First, an explorative analysis was performed in the Leiden EAC data set. In this data set, the tagged SNPs were tested both additively and recessively. Because phase 1 was an

explorative phase, no correction for multiple testing was applied, and SNPs with a *P* value less than or equal to 0.05 were studied in phase 2. For analyses involving the Leiden EAC, Groningen, and Lund data sets, a multivariate normal regression model for longitudinal data was used, with the radiographic score as the response variable. This method is used to analyze all repeated measurements at once and takes advantage of the correlation between these measurements. This model is similar to a linear mixed-effects model, but no random-effects model is added.[26] To model the correlation over time, a heterogeneous first-order autoregressive matrix was used, which assumes a stronger correlation for measurements obtained over a shorter period of time compared with those obtained over a longer period of time. The effect of time was entered as a factor in the model, to capture properly the mean response profile over time.

To test for an association with the rate of joint destruction, an analysis with the SNP and its interaction with time (as a linear variable) in the model was conducted. Adjustment variables were entered based on their univariate association with joint destruction. In the Leiden EAC data set, adjustments were made for age, sex, and the described treatment periods. The Groningen data set was adjusted for age and the period before or during 1990 and the period after 1990, as a proxy for DMARD therapy. The analysis of the Lund data set was adjusted for age only, because sex and treatment were not associated with joint destruction in this data set.

In the Sheffield data set, each patient had a set of radiographs of the hands and feet at one time point. To make the scores comparable with those of the other data sets, the estimated yearly progression rate was calculated.[27] This was achieved by dividing the total Larsen score by the number of disease-years at the time of radiography, resulting in an estimation of the rate of joint destruction. Information regarding disease duration at the time of radiography was available for 391 patients. The SNP association was tested in a linear regression analysis, with the log-transformed estimated yearly progression rate as the outcome variable. No adjustments were applied, because none of the tested variables was univariately significantly associated with joint destruction. The analyses were performed using SPSS version 17.0. In all data sets, the radiographic scores were log-transformed to obtain a normal distribution. Because the analyses were performed on the log scale, the resulting coefficient on the original scale indicates the fold increase in joint destruction per year of follow-up: over a follow-up period of n years, the coefficient increases to the power of n.

In the present study, the power to detect genetic effects is a function of the number of patients and the number of measurements per patient studied. As shown previously, the precision of the estimate increases steadily with increasing numbers of radiographs per patient.[28] All 3 data sets studied to verify the results obtained in phase 1 contained (individually and combined) fewer radiographs than the initial data set. Consequently, the power to replicate findings in each data set individually as well as in the 3 replication data

sets together could be limited due to the large number of radiographs in the discovery data set. Because of differences in study designs, the separate data sets could not be combined in one analysis directly. Therefore, the SNPs in each data set were tested separately, thereby taking advantage of the specific data set characteristics, and a meta-analysis of the results was performed subsequently to determine the association of the SNPs with the rate of joint destruction. Because the parameters in all data sets reflect the relative increase in the rate of joint destruction per year, the estimates of the individual data sets were pooled in a meta-analysis.[29] A fixed-effects meta-analysis[30] with inverse-variance weighting was performed using Stata version 10.1.

RESULTS

Phase 1 (SNP identification)

GZMB was tagged by 16 SNPs, one of which was not analyzed because of a low typing success rate. From the 15 analyzed SNPs, 2 (rs8192916 and rs12433772) were significantly associated with joint destruction in the Leiden EAC data set (Table 2). Patients homozygous for the minor allele of rs8192916 had an average 1.05-fold (range 1.02–1.08- fold) increase in the rate of joint destruction per year compared with the rate in the other patients ($P = 1.2 \times 10^{-3}$) (Figure 2). Because the estimated rate of progression increases by the power of the number of years, a coefficient of 1.05 per year resulted in a 1.41-fold increased rate of joint destruction over 7 years. SNP rs12433772 was highly linked to rs8192916, and because the effect of rs8192916 was more evident at the subsequent time points, this SNP was prioritized for the phase 2 analysis.

Table 2 SNPs in *Granzyme-B* associated with progression of joint destruction in Leiden RA-patients.

SNP	coordinate	Tested model	MAF	Coefficient	95% CI		*P*
rs8192916	24174200	ADD	0.42	1.01	1.00	1.03	0.11
		REC		1.05	1.02	1.08	1.2×10^{-3}
rs12433772	24190515	ADD	0.38	1.02	1.00	1.04	0.02
		REC		1.06	1.03	1.09	2.9×10^{-4}

MAF: minor allele frequency. 95%CI: 95% confidence interval

For the tagging process NCBI build 35, dbSNP b125 was applied. To make all coordinates in the paper consistent (SNPs and probes) NCBI build 36 was used to report the locations.

The coefficient of the additive test represents the relative increase in joint destruction per year per minor allele compared to no minor alleles. The coefficient of the recessive test represents the relative increase in joint destruction per year for two minor alleles compared to no one or no minor alleles on the normal scale. A coefficient of 1.05 per year indicates 5% higher rate of joint destruction; this implies that over a period of 7-years a 41% (1.05^7=1.41) higher rate of joint destruction is achieved.

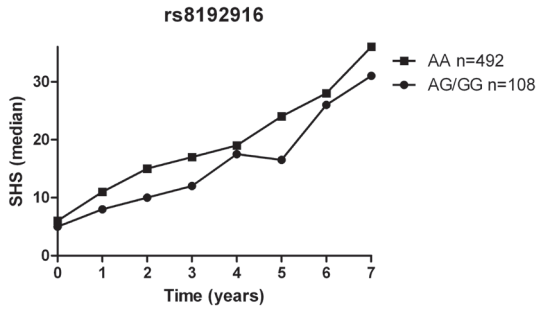

Figure 2 Median Sharp-van der Heijde scores over 7 follow-up years of Leiden RA patients with different rs8192916 genotypes.
Patients homozygous for the minor allele of rs8192916 had 1.05 (1.02-1.08 P=1.2x10^{-3}) fold rate of joint destruction per year than the other patients. This equals a 1.41 fold rate of joint destruction over a follow-up of 7 years.

Phase 2 (meta-analysis)

SNP rs8192916 was tested in a recessive model in all 3 replication data sets. As expected, due to insufficient power of the replication cohorts, the 95% confidence intervals (95% CIs) in each of the 3 cohorts separately all included 1 (Figure 3). An inverse-variance weighting meta-analysis was used to analyze rs8192916 in all 1,418 patients. SNP rs8192916 remained significant (P = 7.8 x 10^{-4}), thereby estimating a higher rate of joint destruction per year in patients homozygous for the minor allele compared with the other patients

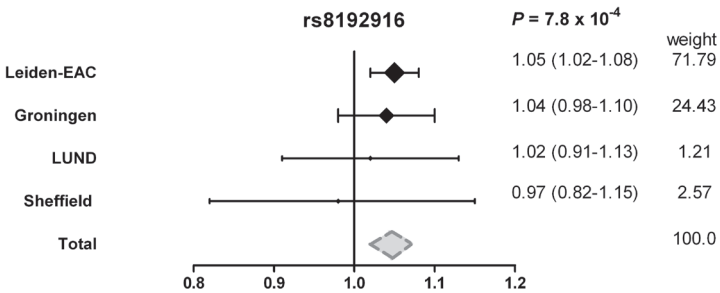

Figure 3 Depicted are the results of the analyses of rs8192916 of all four cohorts and of the final meta-analysis.
The effect sizes are the estimated relative progression rates per year for the presence of twice the minor allele compared to patients with only one or no minor allele.
The meta-analyses are based on a fixed effect model, which are applied to genetic studies to test whether there is statistically significant effect; generalisability of the effect is of less importance. As result of this choice, the effect size of the meta-analyses should be considered carefully. Consequently, this method is less suitable to estimate the effect size overall. Therefore, the estimated effect of the meta-analysis is depicted in gray.

(Figure 3). This association remained significant ($P = 1.2 \times 10^{-2}$) after conservative correction (Bonferroni adjustment) for testing 16 SNPs (the number of SNPs tested in phase 1).

When the association of rs8192916 with the rate of joint destruction was stratified for anti–citrullinated protein antibody (ACPA) status, the association was present in both subgroups (effect size 1.05 [95% CI 1.02–1.08], $P = 1.98 \times 10^{-3}$ and effect size 1.03 [95% CI 0.99–1.08], $P = 5.40 \times 10^{-2}$ for ACPA-negative and ACPA-positive patients, respectively).

Measuring and analyzing *GZMB* expression levels

To further substantiate the observed association of rs8192916 with joint destruction, the effect of rs8192916 on RNA expression was studied by *cis*-eQTL mapping in peripheral blood samples obtained from 1,469 unrelated individuals.[22]

SNP rs8192916 was significantly correlated with RNA expression of 4 different genes: *GZMB, CTSG, CBLN3,* and an unnamed gene with accession no. AK056368 ($P = 1.38 \times 10^{-5}$ to 6.14×10^{-6}) (Table 3). For all 4 genes, a SNP other than rs8192916 was more strongly correlated with RNA expression. To assess whether the SNP associated with joint destruction, rs8192916, had an effect on gene expression independently of the effect of the most strongly associated SNPs, the analyses were subsequently adjusted for the strongest SNP. After this correction was performed, rs8192916 was still associated with RNA expression of the *GZMB* probe ($P = 0.015$) (Table 3).

Table 3 Results of significant correlations of rs8192916 with *cis*-eQTL of RNA in peripheral blood.

SNP	Expressed Gene	Probe midpoint	eQTL significance	FDR	Strongest associating SNP* (*P-value*)	Corrected *P-value*[8]
rs8192916	GZMB	24171665	2.27×10^{-5}	3.03×10^{-5}	rs2236337 (3.98×10^{-119})	0.015
	CTSG	24113935	1.38×10^{-5}	1.38×10^{-5}	rs12878578 (8.91×10^{-63})	0.115
	CBLN3	23967077	2.27×10^{-5}	4.54×10^{-5}	rs2273629 (1.79×10^{-69})	0.057
	AK056368	24016093	6.14×10^{-6}	2.46×10^{-6}	rs12896086 (3.46×10^{-14})	0.752

Depicted information on location of probes is based on NCBI 36.3 build.

* Other SNPs in the region were stronger correlated with the RNA-expression, the strongest are summarized here.

[8] The association of rs8192916 with *cis*-eQTL was corrected for the strongest correlated SNP*.

Rs8192916 was correlated with the RNA-expression of several probes. Four probes, covering four different genes, were significantly correlated to rs8192916. These four correlations were further corrected for the SNPs* that correlated strongest with the RNA-expression.

DISCUSSION

The variance in joint destruction between patients with RA is considerable, and the mechanisms driving these differences are thus far scarcely understood. We performed a candidate gene study to investigate the association of genetic variants in *GZMB* with joint destruction. *GZMB* (14q11.2) was chosen as a candidate gene, because granzyme B is involved in inflammation, and data are emerging that granzyme B could be relevant to joint destruction in RA.[5-8,10,11] We tested the association of SNPs tagging *GZMB* with the rate of joint destruction in one data set and subsequently tested the significant SNP in 3 other data sets. Next, a meta-analysis combining the radiographic data of all 1,418 patients was performed. One SNP, rs8192916 (situated 9 kb upstream of *GZMB*), was observed to associate significantly with progression of joint destruction. The minor allele of rs8192916 was associated with a higher rate of joint destruction. We further observed that carrying this minor allele of rs8192916 was correlated with higher RNA expression of *GZMB* in whole blood. The present study uniquely combines 4 data sets representing patients with a similar European ethnicity who were treated in the prebiologic agent era. Hence, the radiographic progression rate of the patients studied here are more reflective of the natural course of RA compared with that of recently treated patients. In some data sets, patients were included over a wide time span; as treatment strategies changed over time, these patients received different treatments. Because different treatment regimens are associated with our outcome of interest (joint destruction), the analyses were adjusted for treatment, when relevant. Further studies on different patient populations as well as those treated with biologic agents would be informative.

Replication data sets are ideally larger than the initial data set; because the effect sizes in the discovery data set are likely to be upwardly biased, a smaller effect size is expected at a replication stage. Unfortunately, relatively few large prospective data sets exist in which both radiographs and DNA are available for conventionally treated patients. The number of patients and the number of radiographs in each data set separately were insufficient to allow well-powered analyses. Also, the replication data sets combined contained fewer radiographic measurements than were included in phase 1. Consequently, the data available for the phase 2 analysis were expected to be underpowered to replicate findings individually. Therefore, data were summarized in an inverse-variance weighting meta-analysis, which showed a significant result for rs8192916.

To further substantiate the findings for *GZMB*, RNA expression was studied and revealed a strong signal for rs8192916. To confirm that this correlation was not driven by a linked SNP, we sought to identify other SNPs in the region that also associate with RNA expression. SNP rs2236337 had the strongest correlation with *GZMB* expression. However, LD between rs2236337 and rs8192916 was low (r^2 = 0.08). Moreover, the fact that after

correction for this SNP, rs8192916 remained significant suggests that the correlation of rs8192916 with *GZMB* expression is likely independent of the influence of rs2236337.

In the current study, *GZMB* was tagged by pairwise SNP selection, with an MAF of >0.05 and a pairwise r^2 value of >0.8. Inherent to this method, rare variants are not selected. Therefore, based on the results of the current study, we cannot exclude the possibility that other variants in *GZMB* also associate with joint destruction.

Granzyme B, which is released by NK cells and T lymphocytes, can induce cell death in harmful cells, such as those that are virally infected or malignant.[31] Recent findings have suggested a role for granzyme B in RA joint destruction as well; the number of granzyme B–positive cells is increased in synovium as well as among chondrocytes at the site of pannus lesions.[8,32]

The findings of the current study are consistent with those of previous studies. Possibly, the minor variant of rs8291916, or a genetic variant linked to this SNP, induces higher expression of granzyme B, leading to more apoptosis in chondrocytes and therefore more joint destruction. This would explain the previously observed association of higher serum granzyme B levels in patients with erosive disease.[11] Another explanation could be the role of granzyme B in propagation of the inflammatory response; additional studies are needed to determine this.

In conclusion, using a candidate gene approach evaluating patients in 4 different cohorts, we observed associations between rs8192916 and an increased rate of joint destruction in RA and between rs8192916 and expression profiles of *GZMB*. Although evaluation of rs8192916 at the protein level was not performed, these data suggest that carriage of the risk allele may affect the function of *GZMB*.

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