Investigation of Rheumatoid Arthritis Genetic Susceptibility Markers in the Early Rheumatoid Arthritis Study Further Replicates the *TRAF1* Association with Radiological Damage

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ABSTRACT. *Objective.* The *TRAF1* genetic region conferring susceptibility to rheumatoid arthritis (RA) has been reported to associate with radiological damage. We aimed to test RA genetic susceptibility markers for association with a continuous measure of radiological damage over time using longitudinal modeling techniques.

Methods. Sixty-seven RA susceptibility variants were genotyped in 474 patients in the Early Rheumatoid Arthritis Study (ERAS) using Sequenom MassArray technology. Correlation between genetic markers and Larsen score was assessed longitudinally using zero-inflated negative binomial regression to include repeat measurements in the same individual at different timepoints. Genetic markers associated with radiological damage in ERAS were tested using the same modeling techniques on previously published data from the Norfolk Arthritis Register (NOAR).

Results. The single marker associated longitudinally with Larsen score in ERAS (p = 0.02) and in NOAR (p = 0.04) was rs2900180 at the *TRAF1* locus. Analysis of individual timepoints in ERAS showed that rs2900180 displays its effect primarily on the extent of Larsen score early in the disease course. Combined longitudinal analysis of the 2 cohorts suggests further association of several loci with Larsen score (*KIF5A*, *PTPN22*, *AFF3*, *TAGAP*) and therefore a significant accumulation of RA severity markers among RA susceptibility markers (p = 0.016).

Conclusion. The marker rs2900180 is associated with the extent of radiological damage in the ERAS cohort. This represents the second independent study correlating rs2900180 at the *TRAF1* locus with radiological severity in RA. Replication in a large dataset is required to establish the role of other RA susceptibility loci in disease severity. (First Release Dec 15 2012; J Rheumatol 2013; 40:144–56; doi:10.3899/jrheum.121034)

Key Indexing Terms:
GENETIC ASSOCIATION   RADIOLOGICAL DAMAGE   EROSIONS
LONGITUDINAL MODELING   RHEUMATOID ARTHRITIS   *TRAF1*

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Rheumatoid arthritis (RA) susceptibility variants within the genomic interval flanked by the tumor necrosis factor (TNF) receptor-associated factor-1 and complement component 5 genes (*TRAF1/C5*) were first described in 2 independent disease association studies in 2007[1,2]. The studies reported association with 2 different markers in this region: rs2900180 and rs3761847. The markers rs2900180 and rs3761847 are in moderate linkage disequilibrium (LD; $r^2 = 0.69$) and it is not clear which is more closely associated[2]. Following the discovery of this locus, the association with RA susceptibility was confirmed in independent cohorts[3] and by metaanalysis[4,5,6] and is more strongly associated with anticitrullinated peptide antibody (ACPA)-positive RA[7,8].

The *TRAF1/C5* locus variants have since been investigated for correlation with RA outcome. No association has been detected with continuation on methotrexate as a monotherapy[9], response to TNF blockade therapy[10], or premature mortality[11,12]. However, the markers rs10818488 ($r^2 = 0.97$ with rs3761847) and rs2900180 have both been correlated with radiological severity[1,13]. Although the effects at this locus are modest, detection of a relationship between radiological outcome and the *TRAF1* locus in 2 independent studies is intriguing and warrants further investigation in independent cohorts. A recent study replicated the association of rs3761847 with radiological damage in Iceland, but could not replicate this association or the association of rs10818488 in 6 other cohorts originating from different countries[14]. Another recent study showed rs10818488 to be associated with radiological damage in Egyptian patients[15]. However, there have been no published reports investigating rs2900180 in an independent population. Importantly, the various studies above differ in study design, populations, or statistical approach, and most of them are underpowered. Moreover, the definition of radiological damage across studies is heterogeneous. Measures of radiological outcome have been presence of bone erosions and extent of a continuous Larsen or Sharp-van der Heijde score, either cross-sectionally or its progression over time[16].

There are now > 60 single-nucleotide polymorphism (SNP) markers mapping to over 30 loci with confirmed or highly suggestive evidence for association with RA susceptibility[1,2,5,6,17,18,19,20]. Although several studies have investigated non-HLA RA susceptibility markers as putative markers of disease outcome, no convincing genetic marker of erosive disease has yet been identified outside the HLA[16]. In our study, we aimed to test the hypothesis that RA susceptibility markers also modulate disease severity. First, we investigated 67 genetic predictors of RA development, including rs2900180 and rs10760130 ($r^2 = 1$ with rs10818488), as potential determinants of radiological outcome using longitudinal statistical modeling to increase power. Markers with suggestive evidence of association in

the Early Rheumatoid Arthritis Study (ERAS) were analyzed again in previously published data from the Norfolk Arthritis Register (NOAR) using the modeling techniques described here. Longitudinal modeling has the advantage of incorporating all the information on radiological outcome assessed at different timepoints into the analysis and can incorporate multiple measures for the same individual. Second, we combined these data to further increase detection power.

MATERIALS AND METHODS

Patients. ERAS recruited adult patients with RA between 1986 and 1998 from 9 UK centers as described[21]. Briefly, entry criteria included < 2 years of symptoms and no prior treatment with disease-modifying antirheumatic drugs. Clinical and demographic data, collected at baseline assessment, included sex, age at symptom onset, and disease duration at registration. Hand and feet radiographs were taken systematically at baseline, 6 months, and 1 year after registration and yearly thereafter, for 15 years. Radiographs at baseline and yearly thereafter up to Year 9 were scored using the Larsen method[22]. Erosive disease was defined by the presence of at least 1 joint with a Larsen score ≥ 2 (interruption of Corticalis continuity). Radiographs were scored by a single medically qualified researcher (CS) with built-in observer reliability/repeatability mechanisms (intrareader correlation coefficient 0.97), as described[23]. A blood sample was collected at recruitment to permit genetic studies. Only whites in the UK were included in this analysis. ERAS received ethical approval from the West Hertfordshire Local Research Ethics Committee and subsequently from the Caldicott Guardian. Informed consent was obtained from all patients.

NOAR is a primary care-based inception cohort of white UK patients with recent-onset inflammatory polyarthritis (IP) followed prospectively from disease onset to almost 20 years. Details of the NOAR are described elsewhere[13,24]. Briefly, adults aged ≥ 16 years with swelling of 2 or more joints lasting 4 weeks or longer were referred to NOAR. Radiographs of the hands and feet were performed at baseline, Year 1, and Year 2 if required to establish the diagnosis of RA, and systematically at Year 5 for all patients and scored using the Larsen technique. Radiographs were read independently by 2 observers who were blinded to the sequence. In preliminary studies, the intraobserver and interobserver agreements for the presence of erosions were 90% and 81%, respectively[25]. Disagreement on the erosion status was settled by arbitration by a third investigator. Consensus-reading sessions were held to settle minor disagreements on scores. Although no formal interobserver correlation between ERAS and NOAR was performed, the high within-study correlations make a high inter-study correlation likely. The NOAR cohort shares similar characteristics with the ERAS cohort. Ethical approval for the study was obtained (MREC 99/8/84) and informed consent was signed by all patients.

The goal was to identify/replicate genetic markers of future disease severity to guide treatment decision early at disease onset, before American College of Rheumatology criteria for RA were satisfied. The power of the study and the discriminatory capacity of a future genetic test are larger early in the disease course than later; we therefore performed the analysis on IP in NOAR.

Genotyping. SNP markers were genotyped in ERAS patients using Sequenom MassArray technology, in accord with the manufacturer's instructions. Markers were selected from large case-control disease association studies in RA or resultant metaanalyses[1,2,5,6,17,18,19,20]. Patients with > 10% missing genotype data and SNP with < 90% genotyping success rate were excluded prior to analysis as part of quality control procedures. HLA-DRB1 "shared epitope" alleles (HLA-SE: coded as 0, 1, or 2 copies) were detected using sequence oligonucleotide typing, as described[26]. The following HLA-DRB1 genotypes were considered as SE alleles in ERAS or in NOAR: 0101, 0102, 0104, 0105, 0107, 0108, 0110,

0111, 0401, 0404, 0405, 0408, 0409, 0410, 0413, 0416, 0419, 0421, 0423, 0426, 0428, 0429, 0430, 0433, 0434, 0435, 0438, 0440, 0442, 0443, 0445, 1001, 1113, 1126, 1134, 1402, 1409, 1413, 1417, 1419, 1420, 1421, 1429, 1430, 1431, 1432, 1434, 1441, and 1446, 1447, 1448. The genotyping of SNP markers for the NOAR cohort has been published using cross-sectional multivariate analysis[13]. All r[2] values mentioned in this work to qualify the extent of LD between markers have been calculated using the HapMap CEU reference panel.

Longitudinal multivariate analysis. To incorporate multiple records per patient over time into the same model, Larsen score was considered as a time-dependent longitudinal trait. The Larsen score, in particular at early followup, is not normally distributed, with an excess of zero values (nonerosive patients). Consequently, a zero-inflated negative binomial (ZINB) regression model[27] was fitted for every SNP to assess association with Larsen score. This regression model has 2 components: one for the probability that the score is non-zero (susceptibility model), and a separate model for the magnitude of the score, given that it is non-zero (severity model). A given predictor may contribute to either of these models, or both. To allow for the within-subject correlation, a clustered Huber/White/ sandwich estimate of the SE of the model measurements was used[28].

Disease duration at the time of Larsen score measurement was defined as disease duration at registration plus number of years of followup. To incorporate timepoints up to the 5-year followup for all patients, the analysis was performed for disease duration < 7.5 years. To account for change in Larsen score over time, disease duration was included in the regression model. Both linear and quadratic terms in disease duration were included, to allow for nonlinearity in the changes in Larsen score with time. The ZINB regression provides 2 separate point estimates and p values for every SNP, for the susceptibility and severity submodels. The 2 p values were combined and reported as ZINB combined p value. This was not possible for the point estimates, because they have different units. Instead, the marginal effect dy/dx of the model for the average value of x (i.e., the SNP) was calculated and reported as "time averaged effect size" in Larsen units. The modeling was undertaken in each cohort separately and then a combined analysis of both cohorts was performed by pooling observations from the individual cohorts into a unique dataset and adjusting for cohort of origin by fitting terms for the interaction between cohort and disease duration. This accounts for the difference in the velocity of disease progression between the 2 cohorts.

This approach allowed incorporation of all radiographs taken at different timepoints for all patients in every cohort into a single model, and did not require that only timepoints are taken into account, where radiographs have been performed systematically for all patients in a cohort.

Cross-sectional multivariate analysis. Correlation between genetic markers and Larsen score was assessed cross-sectionally at baseline and at subsequent followups with a ZINB model if erosive and nonerosive patients were included in the analysis, or with negative binomial regression if only erosive patients were considered. Logistic regression was used to assess the effect of an SNP on the probability of a subject having erosions. Based on previous experience and because of low counts of homozygotes for the minor allele for most SNP, we assumed, as for the above-mentioned longitudinal analysis, a dominant model for all analyses, i.e., carriage of at least 1 copy of the minor allele versus no copy[12]. Adjustment in cross-sectional analyses was made in every model for symptom duration at baseline.

All analyses were performed using Stata version 10.1 software (StataCorp. 2007, Stata Statistical Software Release 10, StataCorp LP).

Multiple testing. No correction for multiple testing was applied for the p values presented in the results tables. Several tested SNP are in LD and map to the same genetic locus (Appendix 1). The number of independent tests is therefore smaller than 67. There is, however, no consensus on the LD threshold to use for multiple testing corrections. Moreover, under a prior hypothesis of no association, a more stringent correction for multiple testing should be applied than in replication studies. Prior evidence of

association, as is the case for several markers including *TRAF1/C5* markers, does not formally require corrections for multiple testing.

RESULTS

ERAS cohort. Within the first 5 years of followup, nearly three-quarters of the patients in the ERAS cohort developed bone erosions (Figure 1). To model radiological damage as a continuous trait over the years when the vast majority of patients develop erosive disease, correlation between SNP markers and the Larsen score included all timepoints up to a disease duration of 7.5 years. Sixty-seven SNP (Appendix 1) were genotyped in 474 DNA samples from the ERAS cohort. One SNP (rs934734) failed to genotype. Therefore, following quality control, 66 SNP remained available for analysis in 445 samples (94% sample success). Summary cohort characteristics are presented in Table 1.

Longitudinal analysis of radiological outcome in ERAS. There were on average 5.4 Larsen scores (SD 1.7, range 1–8) per patient in ERAS when analysis was limited to a disease duration of 7.5 years. Larsen scores at every timepoint were highly correlated with scores at the previous timepoint, especially later in the disease course, when radiological progression slows down (average Spearman correlation coefficient between subsequent measurements: 0.87). Taking this into account, and assuming a minor allele frequency (MAF) of around 40% (i.e., *TRAF1/C5* markers or SE), the power to detect a time-averaged difference of 2 Larsen units at a significance level of 0.05 between carriers of the minor allele and non-carriers is 34%, using a longitudinal approach. The power rises to 64% for a difference of 3 Larsen units and to 97% for 5 units. The estimated sample size required to detect differences in Larsen scores of 2 units is about 2000 patients, assuming 5 repeated measurements correlated at 0.87 and a MAF around 10% (i.e., *PTPN22*). The shared epitope showed a highly significant association with Larsen score (ZINB combined p value: 5.12E-05) with a strong time-averaged effect size (marginal effect dy/dx): the carriage of at least 1 copy of the shared epitope increased the Larsen score on average by 5.36 Larsen units over the time period considered for analysis (Table 2 and Figure 2). Notably, the shared epitope is already significantly associated with Larsen score at baseline. Results for all tested SNP are in Appendix 1. Three SNP [rs2476601 (*PTPN22*), rs394581 (*TAGAP*), and rs2900180 (*TRAF1*)] displayed a ZINB combined p value < 0.05 for association with Larsen score (Table 2). Those markers were tested using the same modeling technique applied on a previously reported dataset in NOAR to serve as replication.

The predicted values of the Larsen score for carriers versus noncarriers of the minor allele of rs2900180 in ERAS are plotted over time in Figure 2. Unlike the shared epitope, rs2900180 does not significantly affect radiological severity at baseline, but it does influence radiological progression, although the effect size is lower (time-averaged effect size
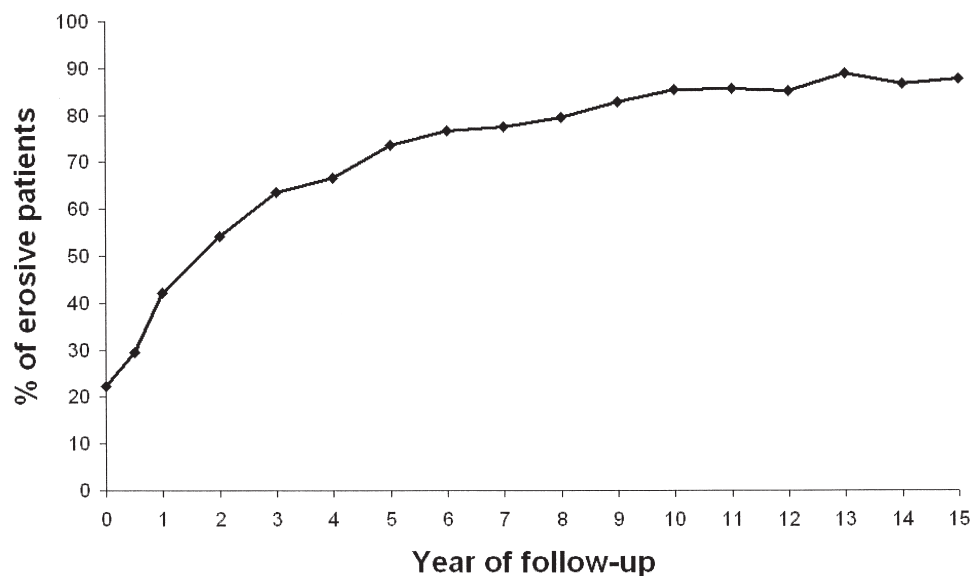
Figure 1. Percentage of erosive patients at every followup in the Early Rheumatoid Arthritis Study.

Table 1. Summary cohort characteristics.

Cohort Characteristics	ERAS	NOAR
No. successfully genotyped patients with radiograph*	445	1446
Ethnicity	UK whites	UK whites
Age at symptom onset, yrs, median (IQR)	56 (45, 65)	56 (44, 67)
Delay from onset to registration, median (IQR), mo	7 (4, 12)	6 (3, 12)
Female, n (%)	293 (66)	980 (68)
HAQ score baseline, median (IQR)	1 (0.5, 1.63)	0.81 (0.25, 1.5)
Larsen score Year 5, median (IQR)	10 (2, 26)	5 (0, 21)
Ever RF-positive, n (%)	389 (87)	628 (44)
Satisfy 1987 ACR criteria during followup, n (%)	427 (96)**	1101 (76)
Followup yrs, mean (SD)	10.2 (4.6)	8.0 (5.1)

* At the time of the study, i.e., when genotyping was performed, ERAS had 1399 white patients with available phenotypic information for epidemiologic studies, but DNA was available for only a fraction of the study population. Of the patients, 445 (32%) had DNA and at least 1 radiographi available, were white, and passed postgenotyping quality control filters. At the time genotyping was performed, NOAR had 3582 patients available for epidemiologic studies, and 1446 patients (40%) satisfied the above conditions. They were therefore available for the longitudinal genetic study presented here. ** Four percent had a clinical diagnosis of RA, as determined by the consultant rheumatologist in charge, although the patients did not formally satisfy the classification criteria for RA. IQR: interquartile range; RF: rheumatoid factor; ERAS: Early Rheumatoid Arthritis Study; NOAR: Norfolk Arthritis Register; HAQ: Health Assessment Questionnaire; ACR: American College of Rheumatology.

dy/dx: 2.96). Adjustment for the shared epitope does not meaningfully affect the results (data not shown), and neither does adjustment for ever-positivity of rheumatoid factor (ZINB combined p value: 0.023; dy/dx: 2.98).

Longitudinal analysis of radiological outcome in NOAR. Detailed cohort characteristics and genotyping have been described[13]; basic cohort characteristics are presented in Table 1 and compared with the characteristics of the ERAS cohort. Both cohorts share many features; however, NOAR recruits patients with IP, while ERAS recruits only patients

with a diagnosis of RA. Therefore, NOAR patients display a lower disease severity for the same disease duration. This does not represent a limitation, because the modeling technique described here accounts for disease duration and for the difference in kinetics between the 2 cohorts, when pooled.

Most (including rs2900180 and rs10760130) but not all of the RA susceptibility markers genotyped in ERAS have been reported in NOAR[13], but analyzed using cross-sectional techniques (Appendix 1). However, the

Chr.	Gene	SNP	Equation	ERAS								NOAR							
				Est.	Lo 95	Up 95	p	N obs	Z obs	Comb p	dy/dx	Est.	Lo 95	Up 95	p	N obs	Z obs	Comb p	dy/dx
1	*PTPN22*	rs2476601	Severity	−0.34	−0.50	−0.13	0.003	2150	684	0.011	−3.60	−0.02	−0.15	0.13	0.784	2089	655	0.667	0.14
			Susceptibility	1.19	0.64	2.24	0.583	2150	684			1.15	0.84	1.58	0.387	2089	655		
6	*TAGAP*	rs394581	Severity	−0.27	−0.42	−0.09	0.007	2155	678	0.024	−3.05	−0.02	−0.14	0.12	0.744	1910	600	0.781	−0.44
			Susceptibility	1.11	0.67	1.86	0.681	2155	678			0.92	0.68	1.22	0.550	1910	600		
6	*HLA-DRB1*	Shared epitope	Severity	0.40	0.03	0.89	0.031	2013	614	5.12 E−05	5.36	0.42	0.23	0.63	6.79 E−07	2102	657	1.46 E−08	4.44
			Susceptibility	3.11	1.76	5.48	8.87 E−05	2013	614			1.52	1.15	2.00	0.003	2102	657		
9	*TRAF1/C5*	rs2900180	Severity	0.39	0.09	0.76	0.007	2161	684	0.024	2.96	0.08	−0.05	0.23	0.256	2168	689	0.037	1.56
			Susceptibility	0.84	0.50	1.41	0.502	2161	684			1.36	1.03	1.79	0.027	2168	689		
9	*TRAF1/C5*	rs10760130	Severity	0.31	0.02	0.68	0.033	2161	684	0.074	2.18	0.07	−0.07	0.23	0.362	2164	689	0.264	1.14
			Susceptibility	0.77	0.43	1.36	0.365	2161	684			1.21	0.90	1.61	0.202	2164	689		

Chr: chromosome; equation, severity/susceptibility: severity/susceptibility components of the ZINB model; est: estimate, expressed as percentage increase in Larsen score for the severity part of the model, or as OR for the susceptibility part of the model; Lo95: lower 95% CI; Up95: upper 95% CI; N obs: total no. observations; Z obs: among the N obs, no. observations with a Larsen score equal to zero; comb p: ZINB combined p value; dy/dx: time-averaged effect size in Larsen units (marginal effect of the model).
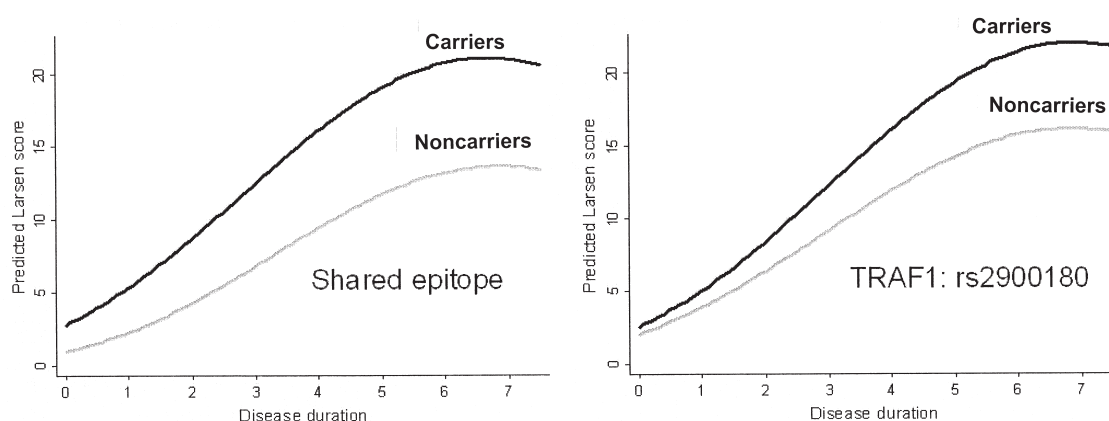
Figure 2. Predicted Larsen score as a function of disease duration and carriage of at least 1 copy of the shared epitope (left panel) or the minor allele of rs2900180 (right panel) in the Early Rheumatoid Arthritis Study. A zero-inflated negative binomial regression model is used, containing disease duration and the shared epitope (left panel) or the minor allele of rs2900180 (right panel) as independent variables. Predicted values for carriers of the shared epitope (left panel) or the minor allele of rs2900180 (right panel) are shown in black and for noncarriers in gray.

power to detect a difference of 2 Larsen units at the 0.05 significance level in NOAR at Year 5 for a MAF around 40% is only 40% when analyzed using a cross-sectional approach. Using a longitudinal approach increases the power to 83% in NOAR, although there were on average 1.5 Larsen scores per patient in NOAR (SD 0.7, range 1–4). This increase of power is mainly achieved by recruiting a significant proportion of patients who did not complete the 5-year followup period or had missing data at 5 years, but had a radiograph taken previously.

Therefore, the NOAR dataset was reanalyzed using longitudinal ZINB modeling including all timepoints up to a disease duration of 7.5 years, to serve as replication for the putative markers of radiological damage identified in ERAS. Only rs2900180 (*TRAF1*) showed an association with Larsen score in both cohorts (Table 2 and Appendix 1). Adjustment for the shared epitope in NOAR did not meaningfully affect the results (data not shown).

Cross-sectional analysis of TRAF1 markers of radiological outcome. Cross-sectional analyses of association between *TRAF1* locus variants and Larsen score or the presence of erosions were performed at baseline and subsequent

followups in ERAS and NOAR. The strongest associations, both with Larsen score and/or erosions, were found early in the disease course at timepoints when roughly half the patients displayed erosive disease. Because of differences in the overall disease severity in ERAS and NOAR, these timepoints are Years 2–3 in ERAS and Year 5 in NOAR (Table 3).

As expected, the results from the continuous part of the ZINB model are consistent with results from the negative binomial regression (NBREG) model, and represent the association of *TRAF1* markers with the extent of Larsen score or radiological damage in erosive patients seen in ERAS (rs2900180, NBREG, Year 3: 43% increase, 95% CI 12%–81%, p = 0.004; rs10760130, NBREG, Year 3: 30% increase, 95% CI 1%–68%, p = 0.046). However, this effect is not significant in NOAR, as reported[13].

The results from the susceptibility part of the ZINB model are consistent with results from the logistic regression model used to predict erosions, and represent the association of *TRAF1* markers with the presence of erosions previously reported in NOAR[13]. However, this effect is not significant in ERAS.

The shared epitope is associated with both the extent of radiological damage and the presence of erosions in ERAS and NOAR (cross-sectional analysis: Appendix 2; longitudinal analysis: Table 2).

Combined analysis in ERAS and NOAR. To improve power, data on 445 patients from ERAS were combined with data from 1446 patients from NOAR and analyzed longitudinally. The power to detect an increase of 1 or 2 Larsen units in the combined cohort for MAF around 40% is 36% or 90%, respectively. For MAF around 10%, a difference of 2 Larsen units will be detected with a power of 80%. The results of the analysis are presented in Appendix 1. Carriage of at least 1 HLA-shared epitope allele was strongly associated with radiographic outcome in each cohort and the strength of the association was increased in the combined analysis (Table 4, ZINB combined p value: 2.81E-10, dy/dx: 4.84). A clear dose effect was observed: dy/dx for 1 copy was 4.26 and for 2 copies, 8.19. Among all SNP tested, 5 loci (*TRAF1*, *KIF5A*, *PTPN22*, *AFF3*, *TAGAP*) showed a ZINB combined p value < 0.05 (Table 4); however, none would remain significant after correction for multiple testing. Nevertheless, the binomial probability of obtaining, by chance, at least 5/30 associated loci at a significance level of 0.05 is 0.016. Consequently, under a prior hypothesis of no association, there is a significant accumulation of RA-susceptibility SNP associated with radiological severity.

Table 3. Cross-sectional analyses of association between *TRAF1/C5* locus variants and Larsen score or the presence of erosions using a zero-inflated negative binomial regression (ZINB) on all patients, a negative binomial regression (NBREG) on erosive patients, or a logistic regression (LOGIT) for the presence of erosions on all patients reveal that TRAF1 markers are associated with erosions in NOAR and with the extent of the radiological damage in erosive patients in ERAS.

Gene, SNP	Cohort	Year	Model	No. Patients	Estimate	Lo95	Up95	p
TRAF1/C5 rs2900180	ERAS	2	ZINB severity	356	0.42	0.05	0.93	0.025
			ZINB susceptibility		0.66	0.32	1.39	0.276
			NBREG	192	0.32	–0.04	0.81	0.092
			LOGIT	435	1.12	0.76	1.64	0.574
		3	ZINB severity	353	0.31	0.04	0.65	0.024
			ZINB susceptibility		1.14	0.58	2.24	0.700
			NBREG	224	0.43	0.12	0.81	0.004
			LOGIT	424	0.86	0.57	1.29	0.467
TRAF1/C5 rs10760130	ERAS	2	ZINB severity	356	0.41	0.03	0.92	0.030
			ZINB susceptibility		0.64	0.28	1.49	0.303
			NBREG	192	0.36	–0.02	0.88	0.069
			LOGIT	435	1.29	0.85	1.96	0.223
		3	ZINB severity	353	0.32	0.02	0.69	0.032
			ZINB susceptibility		1.08	0.52	2.26	0.832
			NBREG	224	0.30	0.01	0.68	0.046
			LOGIT	424	1.08	0.70	1.67	0.716
TRAF1/C5 rs2900180	NOAR	5	ZINB severity	608	0.15	–0.08	0.43	0.223
			ZINB susceptibility		1.88	1.11	3.19	0.020
			NBREG	267	0.06	–0.12	0.27	0.558
			LOGIT	611	1.53	1.10	2.12	0.012
TRAF1/C5 rs10760130	NOAR	5	ZINB severity	607	0.18	–0.07	0.50	0.165
			ZINB susceptibility		1.11	0.64	1.92	0.707
			NBREG	266	0.07	–0.12	0.30	0.513
			LOGIT	610	1.29	0.91	1.83	0.149

NOAR: Norfolk Arthritis Register; ERAS: Early Rheumatoid Arthritis Study; SNP: single-nucleotide polymorphism; Estimate: percentage increase in Larsen score for the severity part of the ZINB model and for the NBREG model, and OR for the susceptibility part of the ZINB model and for the LOGIT model; Lo95: lower 95% CI of the point estimate; Up95: upper 95% CI of the point estimate.

Table 4. Results from the longitudinal zero-inflated negative binomial (ZINB) regression analysis of the Larsen score on the carriage of putative or confirmed rheumatoid arthritis susceptibility single-nucleotide polymorphisms (SNP) in the combined dataset of the Early Rheumatoid Arthritis Study (ERAS) and the Norfolk Arthritis Register (NOAR). Results are shown for SNP with a ZINB combined p value < 0.05 and for the shared epitope. All results are available in Appendix 1.

Chr.	Gene	SNP	Equation	Estimate	Lo95	Up95	ERAS + NOAR p	N obs	Z obs	Comb p	dydx
1	*PTPN22*	rs2476601	Severity	–0.19	–0.31	–0.06	0.005	4239	1339	0.016	–1.80
			Susceptibility	1.16	0.82	1.64	0.390	4239	1339		
2	*AFF3*	rs10865035	Severity	0.02	–0.11	0.18	0.743	4248	1338	0.030	–0.77
			Susceptibility	0.63	0.44	0.89	0.008	4248	1338		
2	*AFF3*	rs1160542	Severity	0.02	–0.11	0.18	0.743	4279	1360	0.018	–0.83
			Susceptibility	0.61	0.44	0.86	0.005	4279	1360		
6	*HLA-DRB1*	Shared epitope	Severity	0.41	0.22	0.64	6.33E-06	4115	1271	2.81E-10	4.84
			Susceptibility	2.03	1.51	2.72	2.77E-06	4115	1271		
6	*TAGAP*	rs394581	Severity	–0.16	–0.27	–0.04	0.011	4065	1278	0.039	–1.81
			Susceptibility	1.00	0.75	1.34	0.977	4065	1278		
9	*TRAF1*	rs2900180	Severity	0.22	0.07	0.40	0.004	4329	1373	0.011	2.25
			Susceptibility	1.10	0.83	1.45	0.509	4329	1373		
12	*KIF5A*	rs775241	Severity	–0.15	–0.27	–0.02	0.029	4324	1370	0.014	–0.95
			Susceptibility	1.42	1.01	1.99	0.045	4324	1370		

Chr: chromosome; equation, severity/susceptibility: severity/susceptibility components of the ZINB model; estimate: expressed as percentage increase in Larsen score for the severity part of the model, or as OR for the susceptibility part of the model; Lo95: lower 95% CI; Up95: upper 95% CI; N obs: total no. observations; Z obs: among the N obs, no. observations with a Larsen score equal to zero; comb p: ZINB combined p value; dy/dx: time-averaged effect size in Larsen units (marginal effect of the model).

Patients carrying at least 1 copy of the minor allele of rs2900180 had on average a 2.25-unit higher Larsen score than homozygotes for the major allele (ZINB combined p value: 0.01). Adjustment for the shared epitope did not meaningfully affect the results (data not shown). No dose effect was observed: dy/dx for 1 copy was 2.59 and for 2 copies 1.40, possibly explained by a loss of power due to lower patient numbers in every category (only 410 homozygotes for the minor allele in the combined dataset).

DISCUSSION

RA susceptibility variants mapping to the *TRAF1/C5* locus on chromosome 9 have previously been investigated for association with radiological outcome in patients with RA[1,13,14,15]. Based on recent evidence, it seems that the originally reported LD block containing rs3761847, rs10818488, and rs10760130 is not associated with radiological severity across several populations[14]. We reported previously an association between a neighboring LD block (rs2900180) and radiological severity[13]. This association had not been reported further. We have found evidence, in a second independent cohort, that this locus (*TRAF1*, rs2900180) is associated with Larsen score, and we have strengthened the evidence for association with this locus using cross-sectional and longitudinal modeling.

Another plausible explanation for the lack of consistency between studies is the heterogeneity in the measurement of radiological damage. For instance, in the Dutch cohort, radiological assessment is made using the Sharp-van der Heijde method, while in NOAR and ERAS, damage is measured using the Larsen score. Second, different outcome measures of radiological damage have been examined. For example, in the Dutch study, investigators focused on progression of damage over time. However, association with extent of Larsen score was investigated in both the NOAR and ERAS studies, with association reported in ERAS but not NOAR. This may reflect chance and power issues, because the sample sizes tested are modest in comparison with studies of disease susceptibility.

The longitudinal modeling techniques presented and used here offer an optimal and more powerful way to analyze time-dependent continuous traits and illustrate the nonlinear progression of radiological damage over time, the early development of erosions, the different kinetics between cohorts, and the need, if cross-sectional analyses are to be performed, to perform them at timepoints when the variance in outcome is maximal. One example would be when half of the cohort is erosive, if the outcome studied is the presence of erosions. Power calculations provided here support the fact that a longitudinal analysis is clearly a more powerful approach compared with a cross-sectional analysis, if radiographs are not performed systematically at every timepoint. The longitudinal analysis appeared to provide greater sensitivity as evidenced by the stronger statistical association observed for the HLA-SE, which served as a positive control for this method. Indeed, the p value for association between the SE and radiological damage in NOAR improves from 1.26E-07 (cross-sectional analysis, Appendix 2) to 1.46E-08 (longitudinal analysis, Table 2), and further to 2.81E-10, when the 2 cohorts are pooled.

APPENDIX 1. Results from the longitudinal zero-inflated negative binomial (ZINB) regression analysis of the Larsen score on the carriage of putative or confirmed RA susceptibility single-nucleotide polymorphisms (SNP). Chr: Chromosome. Equation, severity/susceptibility: severity/susceptibility components of the ZINB model. Est: estimate, expressed as percentage increase in Larsen score for the severity part of the model, or as odds ratio (OR) for the susceptibility part of the model. Lo95: lower 95% CI. Up95: upper 95% CI. N obs: total number of observations. Z obs: among the N obs., number of observations with a Larsen score equal to zero. comb p: ZINB combined p value. dy/dx: time-averaged effect size in Larsen units (marginal effect of the model). NA: not applicable, because the SNP was either not genotyped or did not pass quality control.

Chr.	Gene	SNP	Equation	ERAS								NOAR								ERAS + NOAR							
				Est.	Lo95	Up95	p-value	N obs	Z obs	comb p	dydx	Est.	Lo95	Up95	p-value	N obs	Z obs	comb p	dydx	Est.	Lo95	Up95	p-value	N obs	Z obs	comb p	dydx
1	MMEL	rs10910099	severity	0.01	-0.20	0.28	0.947	2161	684	0.472	-0.61	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			susceptibility	0.72	0.43	1.21	0.220	2161	684			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	PTPRC	rs10919563	severity	0.10	-0.17	0.46	0.489	2105	673	0.689	0.66	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			susceptibility	0.85	0.47	1.54	0.594	2105	673			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	CD2/CD58	rs11586238	severity	-0.08	-0.28	0.17	0.493	2161	684	0.373	-0.18	0.00	-0.12	0.14	0.988	1909	599	0.099	0.82	-0.04	-0.17	0.11	0.578	4070	1283	0.093	0.33
			susceptibility	1.40	0.83	2.37	0.207	2161	684			1.40	1.03	1.90	0.032	1909	599			1.39	1.03	1.89	0.033	4070	1283		
1	FCGR2A	rs12746613	severity	-0.20	-0.46	0.17	0.247	2129	678	0.293	-2.71	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			susceptibility	0.77	0.43	1.40	0.392	2129	678			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	PTPN22	rs2476601	severity	-0.34	-0.50	-0.13	0.003	2150	684	0.011	-3.60	-0.02	-0.15	0.13	0.784	2089	655	0.667	0.14	-0.19	-0.31	-0.06	0.005	4239	1339	0.016	-1.80
			susceptibility	1.19	0.64	2.24	0.583	2150	684			1.15	0.84	1.58	0.387	2089	655			1.16	0.82	1.64	0.390	4239	1339		
1	NHLH2/CASQ2	rs4272626	severity	-0.03	-0.24	0.23	0.806	2161	684	0.203	0.73	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			susceptibility	1.60	0.95	2.68	0.077	2161	684			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1	IL6R	rs7543174	severity	0.03	-0.20	0.32	0.821	2161	684	0.830	0.65	-0.15	-0.26	-0.02	0.023	1907	599	0.048	-1.15	-0.05	-0.19	0.10	0.475	4068	1283	0.477	-0.18
			susceptibility	1.17	0.67	2.07	0.578	2161	684			1.20	0.87	1.66	0.260	1907	599			1.18	0.86	1.64	0.307	4068	1283		
2	STAT4	rs10181656	severity	0.23	-0.03	0.57	0.086	2153	684	0.218	2.41	0.00	-0.12	0.14	0.956	2089	655	0.989	0.09	0.12	-0.03	0.28	0.121	4242	1339	0.282	1.25
			susceptibility	1.07	0.64	1.78	0.799	2153	684			1.02	0.77	1.35	0.895	2089	655			1.04	0.79	1.38	0.768	4242	1339		
2	AFF3	rs10865035	severity	0.09	-0.15	0.41	0.493	2161	684	0.219	-0.11	-0.05	-0.17	0.09	0.500	2087	654	0.057	-1.45	0.02	-0.11	0.18	0.743	4248	1338	0.030	-0.77
			susceptibility	0.59	0.31	1.11	0.103	2161	684			0.68	0.48	0.95	0.023	2087	654			0.63	0.44	0.89	0.008	4248	1338		
2	AFF3	rs1160542	severity	0.12	-0.12	0.43	0.364	2111	671	0.059	-0.21	-0.07	-0.20	0.07	0.289	2168	689	0.137	-1.48	0.02	-0.11	0.18	0.743	4279	1360	0.018	-0.83
			susceptibility	0.48	0.25	0.92	0.026	2111	671			0.77	0.56	1.06	0.105	2168	689			0.61	0.44	0.86	0.005	4279	1360		
2	AFF3	rs11676922	severity	0.19	-0.08	0.54	0.182	2054	650	0.198	1.02	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Chr	Gene	SNP		0.67	0.37	1.24	0.207	2054	650	0.675	0.62	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.01
2	REL	rs13017599	susceptibility	0.09	-0.14	0.39	0.471	2161	684	0.675	0.62	NA	-0.07	-0.19	0.07	NA	0.302	1903	596	0.586	-0.69	NA	0.02	-0.12	0.17	NA	0.829	4064	1280	NA	0.893	0.01
			severity	0.86	0.51	1.46	0.581	2161	684	NA	NA	1.02	0.75	1.37	NA	0.907	NA	1903	596	NA	NA	0.93	0.69	1.27	NA	0.664	NA	4064	1280	NA	NA	NA
2	REL	rs13031237	susceptibility	0.10	-0.13	0.41	0.426	2161	684	0.553	0.59	NA	-0.07	-0.19	0.06	NA	0.275	1906	599	0.551	-0.76	NA	0.02	-0.12	0.18	NA	0.794	4067	1283	NA	0.780	-0.04
			severity	0.81	0.48	1.38	0.436	2161	684	NA	NA	1.01	0.75	1.36	NA	0.951	NA	1906	599	NA	NA	0.90	0.67	1.22	NA	0.504	NA	4067	1283	NA	NA	NA
2	CD28	rs1980422	susceptibility	-0.06	-0.26	0.19	0.627	2161	684	0.867	-0.48	NA	0.15	0.01	0.31	NA	0.041	1893	598	0.124	1.38	NA	0.03	-0.10	0.19	NA	0.640	4054	1282	NA	0.882	0.40
			severity	1.07	0.64	1.77	0.809	2161	684	NA	NA	0.99	0.74	1.32	NA	0.930	NA	1893	598	NA	NA	1.02	0.76	1.37	NA	0.875	NA	4054	1282	NA	NA	NA
2	CTLA4	rs231775	susceptibility	0.22	-0.04	0.56	0.108	2148	683	0.264	1.85	NA	0.01	-0.12	0.14	NA	0.934	2085	655	0.995	0.03	NA	0.10	-0.04	0.27	NA	0.149	4233	1338	NA	0.343	0.93
			severity	0.92	0.54	1.55	0.743	2148	683	NA	NA	0.99	0.75	1.31	NA	0.952	NA	2085	655	NA	NA	0.96	0.72	1.28	NA	0.774	NA	4233	1338	NA	NA	NA
2	CTLA4	rs3087243	susceptibility	-0.16	-0.34	0.07	0.161	2154	681	0.091	-2.92	NA	0.05	-0.08	0.21	NA	0.467	2114	672	0.504	0.16	NA	-0.06	-0.19	0.08	NA	0.374	4268	1353	NA	0.101	-1.39
			severity	0.62	0.35	1.11	0.105	2154	681	NA	NA	0.86	0.64	1.16	NA	0.335	NA	2114	672	NA	NA	0.74	0.54	1.01	NA	0.057	NA	4268	1353	NA	NA	NA
2	STAT4	rs7574865	susceptibility	0.25	-0.02	0.58	0.073	2149	680	0.194	2.49	NA	0.00	-0.12	0.14	NA	0.983	2088	655	1.000	-0.01	NA	0.12	-0.03	0.28	NA	0.115	4237	1335	NA	0.279	1.24
			severity	1.06	0.63	1.76	0.835	2149	680	NA	NA	1.00	0.76	1.32	NA	0.998	NA	2088	655	NA	NA	1.03	0.77	1.36	NA	0.856	NA	4237	1335	NA	NA	NA
3	PXK	rs13315591	susceptibility	0.22	-0.05	0.57	0.120	2155	678	0.210	3.06	NA	-0.05	-0.22	0.16	NA	0.640	1908	597	0.835	-0.26	NA	0.11	-0.07	0.32	NA	0.252	4063	1275	NA	0.317	1.61
			severity	1.44	0.72	2.87	0.305	2155	678	NA	NA	1.10	0.71	1.69	NA	0.671	NA	1908	597	NA	NA	1.25	0.83	1.89	NA	0.290	NA	4063	1275	NA	NA	NA
3	CCR5	rs333	susceptibility	-0.09	-0.35	0.26	0.558	2161	684	0.732	-1.32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			severity	0.86	0.47	1.60	0.645	2161	684	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	PLCL2	rs4535211	susceptibility	-0.15	-0.34	0.10	0.221	2161	684	0.335	-1.16	NA	0.01	-0.13	0.17	NA	0.901	1911	600	0.914	-0.07	NA	-0.08	-0.21	0.08	NA	0.311	4072	1284	NA	0.528	-0.65
			severity	1.28	0.74	2.22	0.377	2161	684	NA	NA	0.93	0.67	1.30	NA	0.679	NA	1911	600	NA	NA	1.09	0.80	1.50	NA	0.590	NA	4072	1284	NA	NA	NA
4	IL2	rs2069778	susceptibility	-0.08	-0.28	0.17	0.487	2161	684	0.783	-0.93	NA	0.05	-0.08	0.21	NA	0.449	2089	655	0.720	0.66	NA	-0.02	-0.15	0.13	NA	0.801	4250	1339	NA	0.967	-0.16
			severity	0.99	0.57	1.71	0.962	2161	684	NA	NA	1.04	0.77	1.41	NA	0.801	NA	2089	655	NA	NA	1.01	0.74	1.38	NA	0.936	NA	4250	1339	NA	NA	NA
4	TNIP2	rs231707	susceptibility	0.00	-0.22	0.29	0.975	2161	684	0.617	0.63	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
			severity	1.32	0.76	2.31	0.327	2161	684	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	IL2/IL21	rs6822844	susceptibility	-0.08	-0.28	0.18	0.531	2161	684	0.822	-0.79	NA	0.07	-0.07	0.23	NA	0.335	2159	688	0.619	0.77	NA	0.00	-0.13	0.15	NA	0.954	4320	1372	NA	0.995	-0.01
			severity	1.01	0.58	1.77	0.969	2161	684	NA	NA	1.02	0.76	1.37	NA	0.907	NA	2159	688	NA	NA	1.01	0.75	1.37	NA	0.930	NA	4320	1372	NA	NA	NA
4	RBPJ	rs874040	susceptibility	-0.04	-0.24	0.21	0.731	2155	682	0.615	-0.96	NA	-0.02	-0.14	0.12	NA	0.745	1903	595	0.850	-0.39	NA	-0.03	-0.16	0.11	NA	0.621	4058	1277	NA	0.531	-0.71
			severity	0.79	0.47	1.32	0.362	2155	682	NA	NA	0.94	0.70	1.25	NA	0.657	NA	1903	595	NA	NA	0.86	0.64	1.16	NA	0.323	NA	4058	1277	NA	NA	NA
5	ANKRD55	rs10040327	susceptibility	0.20	-0.15	0.69	0.304	2160	684	0.575	2.10	NA	0.14	-0.04	0.36	NA	0.141	1902	599	0.227	1.77	NA	0.17	-0.04	0.43	NA	0.119	4062	1283	NA	0.244	1.95
			severity	1.04	0.55	1.97	0.909	2160	684	NA	NA	1.16	0.80	1.67	NA	0.430	NA	1902	599	NA	NA	1.09	0.76	1.57	NA	0.640	NA	4062	1283	NA	NA	NA
5	C5ORF30	rs26232	susceptibility	0.09	-0.15	0.39	0.499	2161	684	0.201	1.79	NA	-0.01	-0.14	0.13	NA	0.845	1909	599	0.941	-0.02	NA	0.04	-0.10	0.20	NA	0.609	4070	1283	NA	0.247	0.92
			severity	1.52	0.92	2.53	0.105	2161	684	NA	NA	1.05	0.78	1.40	NA	0.761	NA	1909	599	NA	NA	1.26	0.94	1.69	NA	0.119	NA	4070	1283	NA	NA	NA
5	ANKRD55	rs6859219	susceptibility	0.05	-0.21	0.38	0.750	2161	684	0.947	0.40	NA	0.04	-0.10	0.20	NA	0.577	1909	599	0.362	0.90	NA	0.04	-0.11	0.22	NA	0.589	4070	1283	NA	0.738	0.65
			severity	0.97	0.56	1.67	0.907	2161	684	NA	NA	1.22	0.89	1.67	NA	0.207	NA	1909	599	NA	NA	1.09	0.79	1.49	NA	0.606	NA	4070	1283	NA	NA	NA
5	IL7R	rs6897932	susceptibility	-0.14	-0.32	0.09	0.208	2153	681	0.441	-1.69	NA	0.05	-0.07	0.19	NA	0.436	2087	653	0.515	0.81	NA	-0.05	-0.17	0.09	NA	0.476	4240	1334	NA	0.761	-0.43
			severity	1.22	0.89	1.67	0.907	2153	681	NA	NA	1.09	0.79	1.49	NA	0.19	NA	2087	653	NA	NA	1.09	0.79	1.49	NA	0.606	NA	4240	1334	NA	NA	NA

The table below is a large landscape-oriented statistical (meta-analysis) table. It has no printed column headers on this page; the values are organized in three analysis blocks of 8 columns each (24 data columns). For each SNP there are two rows ("susceptibility" and "severity"). Blank cells indicate no value printed; NA is printed as shown.

Chr	Gene	SNP	Analysis	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
6	HLA-DRB1	Shared epitope	susceptibility	0.95	0.57	1.60	0.852	2153	681	5.12E-05	5.36	1.12	0.85	1.48	0.428	2087	653	1.46E-08	4.44	1.03	0.78	1.37	0.817	4240	1334	2.81E-10	4.84
6			severity	0.40	0.03	0.89	0.031	2013	614			0.42	0.23	0.63	6.79E-07	2102	657			0.41	0.22	0.64	6.33E-06	4115	1271		
6	TRAF3IP2	rs1043730	susceptibility	3.11	1.76	5.48	8.87E-05	2013	614	0.924	0.36	1.52	1.15	2.00	0.003	2102	657	0.157	0.88	2.03	1.51	2.72	2.77E-06	4069	1283	0.655	0.62
6			severity	0.05	-0.18	0.33	0.717	2161	684			0.02	-0.11	0.16	0.789	1908	599			0.03	-0.10	0.19	0.648	4069	1283		
6	TNFAIP3	rs13192841	susceptibility	0.95	0.57	1.61	0.858	2161	684	0.328	1.11	1.33	0.99	1.79	0.060	1908	599	0.420	-0.97	1.13	0.83	1.53	0.440	4230	1333	0.575	0.07
6			severity	0.03	-0.19	0.31	0.833	2156	682			-0.07	-0.18	0.05	0.241	2074	651			-0.03	-0.15	0.12	0.717	4230	1333		
6	TNFAIP3	rs13207033	susceptibility	1.48	0.88	2.50	0.142	2156	682	0.336	1.08	0.93	0.70	1.23	0.608	2057	645	0.396	-1.01	1.16	0.87	1.54	0.315	4218	1329	0.590	0.05
6			severity	0.02	-0.19	0.30	0.843	2161	684			-0.07	-0.19	0.05	0.230	2057	645			-0.03	-0.15	0.12	0.704	4218	1329		
6	CCR6	rs2301436	susceptibility	1.47	0.87	2.48	0.145	2161	684	0.297	-0.66	0.92	0.70	1.22	0.577	1906	598	0.252	-0.06	1.15	0.87	1.53	0.331	4057	1276	0.839	-0.35
6			severity	-0.14	-0.34	0.13	0.285	2151	678			0.06	-0.09	0.23	0.461	1906	598			-0.04	-0.18	0.12	0.582	4057	1276		
6	TNFAIP3	rs2327832	susceptibility	1.46	0.78	2.72	0.233	2151	678	0.690	-0.17	0.76	0.54	1.08	0.122	2089	655	0.289	-0.23	1.05	0.72	1.51	0.806	4238	1339	0.966	-0.20
6			severity	-0.06	-0.26	0.21	0.648	2149	684			0.03	-0.09	0.17	0.634	2089	655			-0.01	-0.14	0.14	0.855	4238	1339		
6	CCR6	rs3093023	susceptibility	1.22	0.73	2.05	0.451	2149	684	0.190	-0.38	0.80	0.61	1.06	0.127	1898	596	0.225	0.55	0.97	0.73	1.29	0.857	4059	1280	0.723	0.07
6			severity	-0.13	-0.33	0.14	0.321	2161	684			0.11	-0.04	0.28	0.171	1898	596			-0.02	-0.17	0.14	0.780	4059	1280		
6	TAGAP	rs394581	susceptibility	1.55	0.89	2.70	0.120	2161	684	0.024	-3.05	0.82	0.59	1.15	0.249	1910	600	0.781	-0.44	1.14	0.82	1.59	0.444	4065	1278	0.039	-1.81
6			severity	-0.27	-0.42	-0.09	0.007	2155	678			-0.02	-0.14	0.12	0.744	1910	600			-0.16	-0.27	-0.04	0.011	4065	1278		
6	TNFAIP3	rs5029937	susceptibility	1.11	0.67	1.86	0.681	2155	678	0.074	2.56	0.92	0.68	1.22	0.550	2086	653	0.950	0.22	1.00	0.75	1.34	0.977	4234	1329	0.116	1.63
6			severity	0.04	-0.24	0.43	0.809	2148	676			0.03	-0.18	0.31	0.779	2086	653			0.03	-0.17	0.28	0.781	4234	1329		
6	PRDM1	rs548234	susceptibility	3.79	1.18	12.15	0.025	2148	676	0.882	0.02	0.95	0.54	1.66	0.853	1910	599	0.330	1.10	1.88	1.01	3.48	0.046	4060	1277	0.778	0.55
6			severity	0.03	-0.20	0.31	0.829	2150	678			0.08	-0.06	0.23	0.277	1910	599			0.05	-0.09	0.22	0.481	4060	1277		
6	TNFAIP3	rs6920220	susceptibility	0.89	0.53	1.48	0.643	2150	678	0.665	0.59	1.15	0.86	1.54	0.351	2169	687	0.226	-0.33	1.00	0.75	1.35	0.976	4323	1369	0.954	0.13
6			severity	0.01	-0.21	0.28	0.955	2154	682			0.03	-0.10	0.17	0.676	2169	687			0.02	-0.11	0.17	0.793	4323	1369		
6	TNFAIP3	rs6927172	susceptibility	1.27	0.76	2.12	0.369	2154	682	NA	NA	0.79	0.60	1.04	0.090	2081	653	0.316	-0.18	0.98	0.74	1.29	0.861	NA	NA	NA	NA
6			severity	NA	NA	NA	NA	NA	NA	NA	NA	0.03	-0.10	0.17	0.604	2081	653	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	TNFAIP3	rs6933404	susceptibility	NA	NA	NA	NA	NA	NA	NA	NA	0.81	0.61	1.07	0.145	2086	653	0.279	-0.26	NA	NA	NA	NA	NA	NA	NA	NA
6			severity	NA	NA	NA	NA	NA	NA	NA	NA	0.03	-0.09	0.17	0.648	2086	653	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	IRF5	rs10488631	susceptibility	0.06	-0.21	0.42	0.694	2161	684	0.120	-0.76	0.05	-0.11	0.24	0.593	1904	597	0.861	0.40	0.05	-0.12	0.25	0.561	4065	1281	0.166	-0.23
7			severity	0.57	0.32	0.99	0.045	2161	684			0.73	0.69	1.38	0.880	1904	597			0.73	0.52	1.03	0.072	4065	1281		
7	IRF5	rs2004640	susceptibility	0.02	-0.21	0.32	0.859	2142	678	0.150	1.47	-0.01	-0.15	0.14	0.869	1888	593	0.928	0.02	0.01	-0.13	0.17	0.914	4030	1271	0.196	0.78
7			severity	1.72	0.99	2.99	0.053	2143	678			1.06	0.76	1.47	0.721	1888	593			1.34	0.97	1.85	0.072	4030	1271		
7	CDK6	rs42041	severity	0.08	-0.15	0.38	0.520	2143	681	0.072	2.09	0.02	-0.10	0.17	0.722	1897	593	0.915	0.32	0.06	-0.08	0.22	0.445	4040	1274	0.094	1.26
7			susceptibility	1.83	1.06	3.18	0.031		681			1.03	0.77	1.38	0.846	1897	593			1.35	1.00	1.83	0.048	4040	1274		

Note: This is a rotated data table with no printed column headers. Values are reported positionally for three cohort blocks (Cohort 1, Cohort 2, Combined), each with columns: effect (β/OR), lower 95%, upper 95%, p-value, n, cases, and two per-SNP (merged) columns (freq, stat). Merged per-SNP values are shown on the "severity" line.

Chr	Gene	SNP	Analysis	C1:β/OR	C1:L	C1:U	C1:p	C1:n	C1:ca	C1:frq	C1:st	C2:β/OR	C2:L	C2:U	C2:p	C2:n	C2:ca	C2:frq	C2:st	Comb:β/OR	Comb:L	Comb:U	Comb:p	Comb:n	Comb:ca	Comb:frq	Comb:st
8	BLK	rs13277113	severity	-0.05	-0.26	0.20	0.644	2154	681	0.450	0.09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	BLK	rs13277113	susceptibility	1.37	0.82	2.31	0.233	2154	681			NA	NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA		
8	BLK	rs2618476	severity	-0.07	-0.27	0.18	0.533	2149	680	0.404	-0.10	0.07	-0.06	0.22	0.313	1898	594	0.590	0.60	-0.01	-0.14	0.14	0.920	4047	1274	0.664	0.24
8	BLK	rs2618476	susceptibility	1.38	0.82	2.32	0.225	2149	680			0.96	0.71	1.29	0.787	1898	594			1.15	0.85	1.54	0.366	4047	1274		
8	BLK	rs2736340	severity	-0.04	-0.24	0.22	0.728	2161	684	0.382	0.33	0.09	-0.05	0.24	0.216	1905	597	0.369	0.57	0.02	-0.12	0.17	0.797	4066	1281	0.717	0.46
8	BLK	rs2736340	susceptibility	1.43	0.85	2.42	0.175	2161	684			0.89	0.66	1.20	0.438	1905	597			1.12	0.83	1.51	0.447	4066	1281		
9	TRAF1/C5	rs10760130	severity	0.31	0.02	0.68	0.033	2161	684	0.074	2.18	0.07	-0.07	0.23	0.362	2164	689	0.264	1.14	0.18	0.03	0.36	0.021	4325	1373	0.070	1.65
9	TRAF1/C5	rs10760130	susceptibility	0.77	0.43	1.36	0.365	2161	684			1.21	0.90	1.61	0.202	2164	689			0.99	0.74	1.34	0.954	4325	1373		
9	CCL21	rs2812378	severity	-0.01	-0.22	0.27	0.959	2153	682	0.658	-0.61	-0.06	-0.18	0.07	0.340	2164	686	0.392	-0.29	-0.04	-0.16	0.11	0.604	4317	1368	0.857	-0.44
9	CCL21	rs2812378	susceptibility	0.77	0.45	1.34	0.363	2153	682			1.16	0.88	1.53	0.295	2164	686			0.98	0.74	1.29	0.864	4317	1368		
9	TRAF1/C5	rs2900180	severity	0.39	0.09	0.76	0.007	2161	684	0.024	2.96	0.08	-0.05	0.23	0.256	2168	689	0.037	1.56	0.22	0.07	0.40	0.004	4329	1373	0.011	2.25
9	TRAF1/C5	rs2900180	susceptibility	0.84	0.50	1.41	0.502	2161	684			1.36	1.03	1.79	0.027	2168	689			1.10	0.83	1.45	0.509	4329	1373		
9	CCL21	rs951005	severity	-0.13	-0.32	0.13	0.304	2161	684	0.315	-0.63	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	CCL21	rs951005	susceptibility	1.46	0.78	2.74	0.234	2161	684			NA	NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA		
10	IL2RA	rs11594656	severity	0.20	-0.05	0.53	0.128	2111	664	0.149	2.65	0.07	-0.06	0.21	0.332	2134	680	0.209	0.11	0.14	-0.01	0.30	0.068	4245	1344	0.185	1.38
10	IL2RA	rs11594656	susceptibility	1.37	0.81	2.30	0.239	2111	664			1.06	0.62	1.06	0.123	2134	680			1.01	0.77	1.34	0.920	4245	1344		
10	IL2RA	rs2104286	severity	-0.20	-0.37	0.02	0.071	2138	679	0.122	-2.79	-0.05	-0.16	0.09	0.488	2166	689	0.452	-0.83	-0.12	-0.23	0.00	0.057	4304	1368	0.061	-1.78
10	IL2RA	rs2104286	susceptibility	0.79	0.47	1.32	0.362	2138	679			0.87	0.66	1.15	0.322	2166	689			0.83	0.63	1.10	0.193	4304	1368		
10	IL2RA	rs41295061	severity	-0.15	-0.39	0.18	0.336	2161	684	0.392	-2.25	-0.03	-0.20	0.19	0.798	2169	689	0.806	-0.55	-0.09	-0.25	0.10	0.311	4330	1373	0.320	-1.45
10	IL2RA	rs41295061	susceptibility	0.73	0.39	1.37	0.330	2161	684			0.89	0.61	1.31	0.564	2169	689			0.82	0.57	1.17	0.273	4330	1373		
10	PRKCQ	rs4750316	severity	-0.03	-0.24	0.24	0.837	2161	684	0.974	-0.19	-0.02	-0.14	0.11	0.733	2089	655	0.320	0.32	-0.03	-0.15	0.11	0.705	4250	1339	0.636	0.05
10	PRKCQ	rs4750316	susceptibility	1.03	0.58	1.85	0.909	2161	684			1.25	0.93	1.68	0.136	2089	655			1.15	0.84	1.57	0.374	4250	1339		
10	IL2RA	rs706778	severity	0.04	-0.20	0.35	0.761	2083	643	0.679	0.93	-0.02	-0.15	0.13	0.792	1888	592	0.126	0.59	0.01	-0.13	0.17	0.909	3971	1235	0.202	0.74
10	IL2RA	rs706778	susceptibility	1.26	0.72	2.21	0.415	2083	643			1.36	1.01	1.84	0.042	1888	592			1.32	0.97	1.79	0.075	3971	1235		
11	TRAF6	rs540386	severity	0.12	-0.20	0.56	0.505	2161	684	0.673	0.71	-0.01	-0.15	0.14	0.842	1911	600	0.820	-0.39	0.05	-0.13	0.26	0.598	4072	1284	0.621	0.15
11	TRAF6	rs540386	susceptibility	0.81	0.44	1.51	0.510	2161	684			0.91	0.65	1.26	0.560	1911	600			0.86	0.61	1.21	0.384	4072	1284		
12	KIF5A	rs1678542	severity	-0.07	-0.27	0.18	0.530	2161	684	0.668	-1.16	-0.12	-0.23	0.00	0.052	2161	689	0.151	-1.24	-0.10	-0.21	0.04	0.148	4322	1373	0.314	-1.19
12	KIF5A	rs1678542	susceptibility	0.85	0.51	1.42	0.538	2161	684			1.04	0.79	1.36	0.791	2161	689			0.94	0.72	1.25	0.686	4322	1373		
12	SH2B3	rs3184504	severity	0.23	-0.05	0.59	0.120	2066	666	0.297	2.12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
12	SH2B3	rs3184504	susceptibility	1.01	0.59	1.73	0.959	2066	666			NA	NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA		
12	KIF5A	rs775241	severity	-0.24	-0.43	0.02	0.064	2157	684	0.159	-2.35	-0.08	-0.20	0.06	0.259	2167	686	0.012	0.34	-0.15	-0.27	-0.02	0.029	4324	1370	0.014	-0.95
12	KIF5A	rs775241	susceptibility	1.16	0.64	2.11	0.622	2157	684			1.67	1.17	2.39	0.005	2167	686			1.42	1.01	1.99	0.045	4324	1370		
14	BATF	rs7155603	severity	0.11	-0.14	0.43	0.414	2094	656	0.504	1.60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14	BATF	rs7155603	susceptibility	1.24	0.72	2.14	0.432	2094	656			NA	NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA		

The combined analysis, including ERAS and NOAR data, suggests several other SNP markers of severity. A replication of those results is fundamental before any conclusion can be drawn. It has been reported that the *CD40* gene susceptibility risk allele confers protection for disease severity[29]; however, we found no evidence for association in the current datasets. Interestingly, the combined analysis identifies a total of 5 non-HLA loci of interest with a noncorrected p value for association with the Larsen score < 0.05: *TRAF1*, *KIF5A*, *PTPN22*, *AFF3*, *TAGAP*. This represents a significant accumulation of RA susceptibility SNP associated with radiological severity (p = 0.016).

Limitations of our study include the lack of ACPA data in the ERAS samples, which precluded an estimation of the effect of *TRAF1* variants in determining radiological outcome above that accounted for by ACPA positivity. It should also be noted that no correction for multiple testing was applied in the analysis. However, if a Bonferroni corrected p value were applied to account for the number of loci tested, then association with the rs2900180 *TRAF1* variants would not remain significant at the corrected threshold (p = 0.002). Further replication of these findings will therefore be required.

The lack of association between outcome and the majority of markers tested could be related to modest sample size and lack of statistical power. Alternatively, markers of RA susceptibility may not in general have the dual effect of modulating disease course. Large, multicenter collaborations are likely needed to address this issue. A well-powered, dedicated genome-wide study of disease outcome in RA remains to be reported but could identify clinically useful markers of outcome.

We report association of the rs2900180 at the *TRAF1* locus with radiological damage in a second, independent cohort of patients with RA. Incorporation of longitudinal analysis techniques and combining data with that from a previous UK series increased the evidence for association with the locus still further. This confers reasonable evidence that rs2900180 is a true marker of disease severity.

ACKNOWLEDGMENT

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Chr	Gene	SNP	Analysis	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23
18	PTPN2	rs7234029	severity	-0.15	-0.33	0.08	0.195	684	0.310	-1.13	0.00	-0.13	0.16	0.967	1909	598	0.992	0.08	-0.08	-0.20	0.07	0.266	4070	1282	0.410	-0.55
18	PTPN2	rs7234029	susceptibility	1.28	0.74	2.21	0.386	684			1.02	0.74	1.40	0.908	1909	598			1.14	0.83	1.57	0.426	4070	1282		
18	CD226	rs763361	severity	-0.05	-0.27	0.23	0.694	684	0.311	-1.53	-0.02	-0.15	0.13	0.741	2168	688	0.894	-0.37	-0.04	-0.17	0.12	0.639	4329	1372	0.305	-0.92
18	CD226	rs763361	susceptibility	0.62	0.33	1.17	0.144	684			0.95	0.70	1.30	0.757	2168	688			0.79	0.58	1.09	0.149	4329	1372		
20	CD40	rs4239702	severity	0.07	-0.16	0.36	0.607	684	0.291	-0.19	0.04	-0.09	0.18	0.559	2170	689	0.828	0.31	0.05	-0.08	0.20	0.484	4331	1373	0.318	0.05
20	CD40	rs4239702	susceptibility	0.68	0.41	1.12	0.132	684			0.97	0.74	1.27	0.811	2170	689			0.82	0.63	1.09	0.169	4331	1373		
20	CD40	rs4810485	severity	0.15	-0.10	0.47	0.253	684	0.268	0.80	0.01	-0.11	0.15	0.876	2154	653	0.537	-0.29	0.08	-0.06	0.24	0.289	4231	1335	0.176	0.25
20	CD40	rs4810485	susceptibility	0.74	0.44	1.22	0.233	684			0.85	0.65	1.13	0.266	2154	653			0.80	0.60	1.06	0.115	4231	1335		
21	UBASH3A	rs11203203	severity	-0.08	-0.28	0.18	0.496	684	0.780	-1.00	0.12	-0.03	0.29	0.109	1860	581	0.131	0.65	0.01	-0.13	0.17	0.916	4015	1263	0.705	-0.21
21	UBASH3A	rs11203203	susceptibility	0.96	0.58	1.60	0.881	684			0.81	0.59	1.10	0.175	1860	581			0.88	0.65	1.19	0.404	4015	1263		
21	UBASH3A	rs3788013	severity	-0.07	-0.29	0.21	0.572	684	0.619	-1.26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	UBASH3A	rs3788013	susceptibility	0.81	0.48	1.38	0.445	684			NA	NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA		
22	C1QTNF6	rs229541	severity	0.09	-0.15	0.39	0.499	684	0.759	1.05	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
22	C1QTNF6	rs229541	susceptibility	1.08	0.64	1.83	0.772	684			NA	NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA		
22	IL2RB	rs3218258	severity	-0.14	-0.32	0.09	0.205	684	0.447	-1.55	0.03	-0.09	0.18	0.602	2160	686	0.477	0.73	-0.06	-0.18	0.07	0.357	4321	1370	0.531	-0.43
22	IL2RB	rs3218258	susceptibility	1.03	0.62	1.70	0.915	684			1.16	0.88	1.52	0.291	2160	686			1.10	0.84	1.45	0.488	4321	1370		
22	IL2RB	rs743777	severity	-0.05	-0.25	0.21	0.672	684	0.778	-0.21	0.02	-0.10	0.16	0.719	2089	655	0.535	0.61	-0.02	-0.14	0.13	0.808	4246	1339	0.562	0.18
22	IL2RB	rs743777	susceptibility	1.16	0.70	1.94	0.557	684			1.16	0.88	1.52	0.303	2089	655			1.16	0.88	1.54	0.291	4246	1339		

APPENDIX 2. Results from the cross-sectional zero-inflated negative binomial (ZINB) regression analysis of the Larsen score on the carriage of at least 1 copy of the shared epitope single-nucleotide polymorphism (SNP).

Gene, SNP	Cohort	Year	Model	No. Patients	Estimate	Lo95	Up95	p	Comb p
HLA-DRB1	ERAS	3	ZINB severity	327	0.36	0.02	0.83	3.85 E-02	6.38 E-05
Shared epitope			ZINB susceptibility		4.43	2.02	9.70	1.99 E-04	
			NBREG	207	0.46	0.04	1.05	3.02 E-02	—
			LOGIT	394	2.51	1.55	4.05	1.73 E-04	
	NOAR	5	ZINB severity	589	0.84	0.46	1.31	1.99 E-07	1.26 E-07
			ZINB susceptibility		1.35	0.80	2.28	2.55 E-01	
			NBREG	260	0.29	0.06	0.56	1.00 E-02	—
			LOGIT	593	2.50	1.76	3.54	2.55 E-07	

Est: estimate, expressed as percentage increase in Larsen score for the severity part of the model, or as OR for the susceptibility part of the model; Lo95: lower 95% CI; Up95: upper 95% CI; comb p: ZINB combined p value; NOAR: Norfolk Arthritis Register; ERAS: Early Rheumatoid Arthritis Study; NBREG: negative binomial regression; LOGIT: logistic regression.

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