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Dense Genotyping of Immune-Related Regions Identifies Loci for Rheumatoid Arthritis Risk and Damage in African Americans

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More than 100 risk loci for rheumatoid arthritis (RA) have been identified in individuals of European and Asian descent, but the genetic basis for RA in African Americans is less well understood. We genotyped 610 African Americans with autoantibody-positive RA and 933 African American controls on the Immunochip (iChip) array. Using multivariable regression, we evaluated the association between iChip markers and the risk of RA and radiographic severity. The single nucleotide polymorphism (SNP) rs1964995 (odds ratio = 1.97, $p = 1.28 \times 10^{-15}$) near *HLA-DRB1* was the most strongly associated risk SNP for RA susceptibility; SNPs in *AFF3*, *TNFSF11* and *TNFSF18* loci were suggestively associated ($10^{-4} < p < 3.1 \times 10^{-6}$). Trans-ethnic fine mapping of *AFF3* identified a 90% credible set containing previously studied variants, including rs9653442, rs7608424 and rs6712515, as well as the novel candidate variant rs11681966; several of these likely influence *AFF3* gene expression level. Variants in *TNFRSF9*, *CTLA4*, *IL2RA*, *C5/TRAF1* and *ETS1* – but no variants within the major histocompatibility complex – were associated with RA radiographic severity. Conditional regression and pairwise linkage disequilibrium (LD) analyses suggest that additional pathogenic variants may be found in *ETS1* and *IL2RA* beyond those found in other ethnicities. In summary, we used the dense genotyping of the iChip array and the unique LD structure of African Americans to validate known risk loci for RA susceptibility and radiographic severity, and to better characterize the associations of *AFF3*, *ETS1* and *IL2RA*.

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by synovial joint inflammation, with disease phenotype ranging from mild joint involvement to severe joint destruction and permanent disability (1). The factors responsible for RA heterogeneity are poorly understood, but both genetic and environmental factors contribute to its pathogenesis and clinical expression. Most RA patients have serum autoantibodies, such as rheumatoid factor (RF) or anticyclic citrullinated peptide antibody (ACPA), which can be present before the onset of clinically relevant disease (2) and are associated with radiographic severity (3).

To replicate and fine map risk loci identified in genome-wide association studies (GWAS) of autoimmune and inflammatory disorders such as RA, the Immunochip Consortium designed the Immunochip (iChip), a custom Illumina Infinium high-density array that has been used to study RA in patients of several racial and ethnic backgrounds (4–8). Using the iChip and many other arrays, 100 RA risk loci of genome-wide significance ($p < 5 \times 10^{-8}$) have been identified in individuals of European and

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Asian ancestry, including *HLA-DRB1, PADI4, PTPN22* and *CTLA4* (9,10). However, there is a paucity of genetic association data on RA in African Americans. Many of the genetic influences on RA are similar among those of European ancestry and African Americans (11). However, there are important differences; for instance, polymorphisms in *CCR6*, *TAGAP* and *TNFAIP3* have discordant odds ratios (ORs) compared with those reported in European RA patients (11). Furthermore, the *PTPN22* risk allele containing rs2476601, which has the highest effect size on RA susceptibility of any locus outside the major histocompatibility complex (MHC) in European populations, is essentially absent from the Yoruban population and is present in Asian and African American populations in low frequency (12).

HLA-DRB1 alleles encoding the shared epitope (SE) (13) have the strongest association with RA in Europeans and Asians. In addition to their role in susceptibility of RA, *HLA-DRB1* SE alleles are associated with erosive disease (14) and mortality in Europeans (15,16). Our group has shown that 43% of African Americans with RA have at least one *HLA-DRB1* SE allele compared with ~60–70% of Europeans with RA (17). At the level of *HLA-DRB1* amino acid residues and their association with RA susceptibility, there are both similarities and major differences between Europeans and African Americans. The valine residue at position 11, as found in Europeans, is most strongly associated with RA in African Americans (18). However, an aspartic acid residue at position 11, indicative of the classical allele *09:01, confers a two-fold increased risk of RA in African Americans and is also associated with RA in Koreans (19), but not in individuals of European ancestry. After conditioning on residue substitutions at position 11, amino acid positions 71 and 74 are not significantly associated with RA in African Americans, as they are in Europeans (18).

Subphenotypes (ie, disease subtraits) (20) are frequently more heritable than the complex disease traits of which they are a part (21). In addition, genetic association studies on specific subphenotypes tend to focus on less heterogeneous patients than studies on overall disease susceptibility. Radiographic severity is a characteristic RA subphenotype, with an estimated heritability between 45% and 58% (22). Genetic influences on radiographic severity have been examined in several ethnic groups (23–31), and ~30 risk loci have been identified in European and Asian populations, including *CXCR5*, *AFF3*, *C5-TRAF1*, *IL2RA*, *IL6*, *IL10* and *FCRL3*. Typically, these studies have included smaller numbers of participants than studies on susceptibility of RA, and the definition of radiographic severity has not been uniform, which may limit statistical power and complicate replication. Finally, few studies have addressed radiographic severity of RA in African Americans.

The objective of our study is to investigate the associations of known autoimmune disease risk loci with RA and its radiographic severity in African Americans. In view of the heterogeneity of disease associations among ethnic groups, shorter haplotype blocks and differences in allele frequency in African Americans, we hypothesize that fine mapping will identify differences in the genetic architecture of RA in African Americans compared with other ethnicities. Strengths of this study include analysis of the largest group of African Americans with RA currently available in the world, with accompanying high-quality radiographic outcomes data (34). A major goal of research into the genetics of complex diseases is to identify pathogenic variants that produce disease associations. In view of this, we draw on available data from association testing of >100,000 Asians and Europeans and use cutting-edge algorithms to prioritize genetic risk variants in African Americans with RA in the *AFF3* locus. This study represents an important addition to the literature on the genetics of RA, which has primarily involved participants of Asian and European descent.

MATERIALS AND METHODS

Study Population

The CLEAR (Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis) study enrolled African Americans with RA of <2 years' disease duration (CLEAR I), African Americans with RA irrespective of disease duration (CLEAR II) and African American healthy controls, as previously described (17). Participants were enrolled at five academic sites: University of Alabama at Birmingham (coordinating center); Grady Hospital/Emory University, Atlanta, Georgia; University of North Carolina, Chapel Hill; Washington University, St. Louis, Missouri; and Medical University of South Carolina, Charleston. CLEAR controls were African Americans without rheumatic diseases who were matched (as a group) by age, sex and geographic location to CLEAR RA patients. The Institutional Review Boards of the participating institutions approved human subject research protocols. Biologic specimens and patient information, including sociodemographic characteristics, medical history, medications and disease activity measures, were collected (17). The majority of CLEAR participants were ACPA-positive, as previously reported (32). Of the 837 African American healthy controls included for analysis in the current study, 404 were from CLEAR and 433 were from the Birmingham, Alabama, area (33).

Radiographs of hands/wrists and feet were obtained at the CLEAR enrollment visit for participants with RA and assigned a modified total Sharp score (mTSS) (range 0–448) using the modified Sharp/van der Heijde method (34). Scoring was performed using state-of-the-art methods under the auspices of Désirée van der Heijde, a world expert in quantitative assessment of radiographs in rheumatic diseases (34). Furthermore, mTSS scores for participants from CLEAR I and CLEAR II have been validated extensively (35,36).

Sample Genotyping

Genotyping was carried out using the iChip array at the Feinstein Institute for Medical Research in Manhasset, New York. Genotype clustering was performed using the GenTrain2 clustering algorithm. Genotype calling was performed with the genotyping module of the GenomeStudio data analysis software package.

Quality Control

Rigorous quality control procedures were employed, including checks for gender inconsistency, relatedness (duplicates and first- or second-degree relatives) and ethnic outliers. The sample call rate threshold was 95%. The marker call rate was >98.5% across all SNPs, after removing low-quality SNPs and rare SNPs, those with minor allele frequency (MAF) <5% and SNPs out of Hardy-Weinberg equilibrium (using control samples only, using p value $>1 \times 10^{-5}$).

Association Testing of iChip Markers with RA Susceptibility

Of 610 RA cases, 593 (97%) were autoantibody-positive (defined as positive for either RF or anti-CCP antibody tests) and were included in the analysis of RA susceptibility. Multivariable logistic regression was used to evaluate the association between iChip markers and autoantibody-positive RA. Sex and European admixture proportion (calculated using Eigenstrat v6.0) (17) were included as covariates. Two-sided p values are reported, except as noted for trans-ethnic fine mapping of the *AFF3* locus (see Trans-ethnic fine mapping of the *AFF3* locus section). To adjust for variability due to *HLA-DRB1* in the extended MHC locus (Chr6:26,000,000–34,000,000), we fit a model accounting for the variability of all four-digit *HLA-DRB1* SE alleles. LocusZoom plots were used to display the fine mapping results graphically (37).

Association Testing of iChip Markers with RA Severity

The modified total radiographic scores (mTSS) were overdispersed in the CLEAR cohort, with a high proportion of individuals having no erosions or joint space narrowing (mTSS = 0): 156 of 230 CLEAR I participants (67.8%) and 150 of 365 CLEAR II participants (41.1%) (see Supplementary Figure S1). We assessed several count regression models and found that the zero-inflated negative binomial model had the best fit for the data, likely due to the high proportion of participants without damage (mTSS = 0). Thus, we used this method to evaluate the association of genetic markers with radiographic severity (under an additive genetic model). Association testing was carried out using the PSCL package in R (38) after adjusting for body mass index, sex, smoking status, percent European admixture (see [17] for details) and disease duration (in months) as covariates. Due to the inclusion criteria, disease duration was much shorter in CLEAR I (early RA) (median 1.01 years; interquartile range 0.57–1.52 years) than in CLEAR II (any disease duration) (median 9.25 years; interquartile range 3.42–17.75 years). Using a square root transformation for disease duration improved the model fit and reduced genomic inflation ($\lambda_{GC} = 1.10$) compared with a model using untransformed disease duration. After removal of the SNPs in the extended MHC and other associated loci, the λ_{GC} value was further reduced to 1.04 (See quantile-quantile plot in Supplementary Figure S2).

Trans-Ethnic Fine Mapping of the *AFF3* Locus

We conducted trans-ethnic fine mapping of the *AFF3* locus, combining our RA susceptibility data with those from a previously published large trans-ethnic meta-analysis (10). To accomplish this, we: (1) aligned reference and alternate alleles from all Asian, European and CLEAR populations to match those from the 1000 Genomes project (39); (2) generated LD matrices either from our genotyping data (African Americans) or from the 1000 Genomes project (Asian and European populations; data from Okada *et al.*) (10); (3) annotated SNPs from all three ethnicities using 8,138 genomic annotations (for example, DNAse hypersensitivity, enhancer markings and so on) provided with the PAINTOR3 algorithm; and (4) trimmed these to the top five uncorrelated annotations (correlation coefficient <0.10), excluding the annotations with lower Bayes factors.

We then confirmed the algorithm was working properly by examining the results it produced in RA loci in which the causal variant was known. For instance, we generated a posterior probability of 1.0 for rs2476601 in *PTPN22* in Europeans with RA. Following this, we calculated the posterior probability that each variant in the *AFF3* locus was pathogenic using PAINTOR3 (39), which assigns a probability ranging from 0 (very unlikely) to 1 (highly likely). We ran the algorithm using genetic data from all three populations, and defined a "90% credible set" for candidate pathogenic variants as previously reported (40) (see Table 3). Although PAINTOR3 is capable of modeling more than one causal variant per locus, in this study we conducted trans-ethnic fine mapping under the assumption of one causal variant.

Calculation of Number of Effective Markers for the iChip Array

Because the iChip contains many variants concentrated in specific loci and in LD with one another, the number of independent tests is much smaller than the actual number of variants genotyped. Estimates of the number of effective markers for custom genotyping arrays such as the iChip vary widely, between 2,800 and 60,000 LD-independent markers (41–43). To find independent SNPs, we used Plink (44), as previously utilized for iChip data (45), and found 16,154 LD-independent SNPs. We thus defined an iChip-wide statistical significance threshold as 0.05 divided by 16,154 LD-independent SNPs, or $p = 3.1 \times 10^{-6}$, similar to previous reports. We report any variants having $p < 1 \times 10^{-4}$ as showing suggestive statistical associations (for both susceptibility and severity).

Table 1. Demographic, clinical, genetic and radiographic characteristics of African American participants with RA and healthy controls from the CLEAR registry.

	Cases		Controls	
Baseline characteristics	CLEAR I N = 233	CLEAR II N = 360	CLEAR I N = 139	CLEAR II N = 265
Age in years, mean (SE)	50.0 (13.0)	56.0 (11.8)	48.1 (12.4)	57.3 (8.7)
Sex (female), %	82.7	85.0	75.5	72.0
Disease duration in months, mean (SE)	12.9 (7.1)	114.0 (119.2)	–	–
Body mass index, mean (SE)	31.4 (7.8)	–	31.7 (7.6)	–
Global European admixture estimate, mean (SE)	0.17 (0.09)	0.16 (0.10)	0.16 (0.09)	0.17 (0.10)
Number of tender joints, median (IQR 25–75)	4.0 (1.0–12.0)	4.0 (1.0–9.5)	–	–
Number of swollen joints, median (IQR 25–75)	3.0 (1.0–7.0)	4.0 (1.0–10.0)	–	–
Medications				
Biologics ever used (%)	4.4	–	19.5	–
Other DMARDs (%)	81.4	–	87.1	–
Methotrexate, current use (%)	63.9	60.3	–	–
Radiographic score, mean (SE)				
Joint-erosion score	1.6 (4.3)	10.7 (18.5)		
Joint-narrowing score	2.1 (5.7)	18.1 (27.7)		
Total score	3.7 (9.4)	28.8 (44.1)		

SE: standard error; IQR: interquartile range; DMARD: disease-modifying anti-rheumatic drug.

All supplementary materials are available online at www.molmed.org.

RESULTS

Following quality control procedures, 100,268 SNPs with MAF >0.05 were available for analysis in 610 RA cases and 837 healthy controls (as stated in Materials and Methods, 593 [97%] were autoantibody-positive and were included in subsequent analyses). The demographic characteristics of African Americans from the CLEAR registry included in this study are presented in Table 1. Characteristics of Birmingham controls did not differ significantly from the CLEAR registry with respect to sex, European admixture proportion or other variables (17).

Association between iChip Markers and RA Susceptibility

We evaluated the association between iChip markers and RA using logistic regression and adjusting for the proportion of overall European admixture. We observed seven non-HLA loci suggestively associated with RA (defined as $p < 10^{-4}$); the lead SNP (ie, the most strongly associated) at each locus is shown in Table 2. As expected, the markers with the strongest association with RA were found in the MHC region (Figure 1A). We identified rs1964995 in *HLA-DRB1* as the variant with strongest association with RA (OR = 1.97, $p = 1.28 \times 10^{-15}$).

We performed a conditional analysis of the variation contained within the extended MHC region as previously described by Raychaudhuri *et al.* (46). As shown in Figure 1B, conditioning on the *HLA-DRB1* alleles substantially attenuated the strength of association of other variants within the extended MHC region. rs3134792 near *HLA-B* displayed an OR of 2.01 (95% confidence interval [CI] = 1.42–2.88; $p = 9.92 \times 10^{-5}$) for the association with RA susceptibility after conditioning. This effect size and direction of effect are consistent with those reported for amino acid position 9 of HLA-B in Europeans (OR = 2.12, CI = 1.89–2.38). No variants in *HLA-DPB1* were associated with RA after controlling for the *HLA-DRB1* alleles. However, the direction of effect and ORs measured in this locus were similar to those found in studies of European populations. Specifically, although above the threshold for statistical significance, rs9277357 had an OR of 1.34 (95% CI = 1.14–1.59; $p = 5.39 \times 10^{-4}$), which is consistent with that previously reported for amino acid position 9 in *HLA-DPB1* (OR = 1.40, CI = 1.31–1.50).

Table 2. Variants outside the HLA region associated with autoantibody-positive RA in African Americans at $p < 10^{-4}$.

rsID	Chr	Position	A1[a]	OR	95% CI	*P* value	Nearest genes
rs61828386	1	172863647	G	0.69	0.58–0.82	1.79×10^{-5}	*TNFSF18, FASLG*
rs67164098	2	68556131	A	1.67	1.31–2.13	4.65×10^{-5}	*CNRIP1*
rs11681966	2	100759457	C	1.50	1.23–1.78	4.04×10^{-5}	*AFF3*[b]
rs10758368	9	36310778	A	0.70	0.58–0.83	5.48×10^{-5}	*RNF38*
rs9533119	13	43049426	A	1.36	1.17–1.59	6.32×10^{-5}	*TNFSF11*
rs2934178	15	48218221	C	0.70	0.59–0.82	2.96×10^{-5}	*SEMA6D*

Chr: chromosome.
[a]The test allele and minor allele for this the study.
[b]Indicates a validated risk locus for RA.

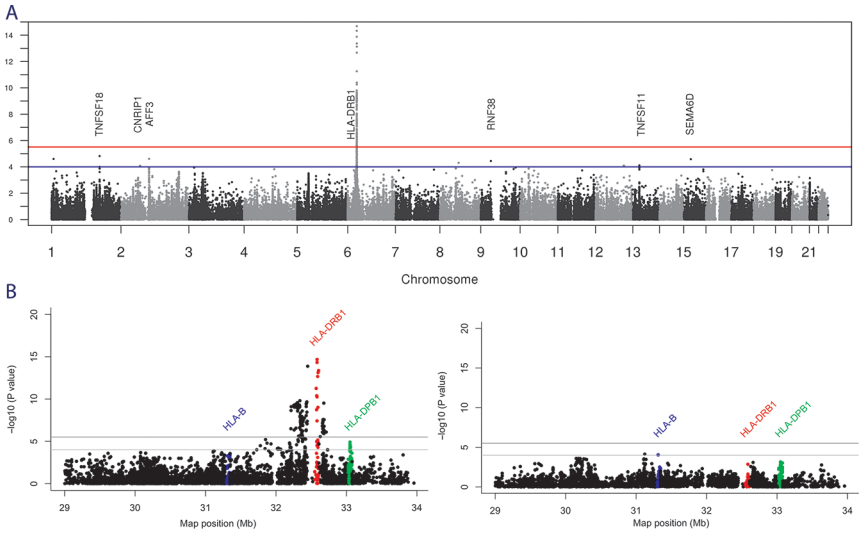

Figure 1. (A) Manhattan plot of the association of iChip variants with autoantibody-positive RA in African Americans. The x-axis indicates chromosome and position, the y-axis indicates association strength –log(p). The blue line illustrates suggestive statistical association ($p = 1*10^{-4}$). The red line illustrates iChip-wide level of statistical significance ($p = 3.1*10^{-6}$). (B) Conditional analysis in the extended MHC region (chr6:26,000,000-34,000,000). Axes have the same meanings as in (A). Red, green and blue dots are SNPs in the regions immediately surrounding *HLA-DRB1*, *HLA-DPB1* and *HLA-B*, respectively. The left panel shows the association summary statistics before conditioning on *HLA-DRB1* alleles. The strongest association maps to *HLA-DRB1*. The right panel shows the locus after conditioning on *HLA-DRB1* 4-digit alleles.

We performed more detailed analysis on *AFF3* (see Materials and Methods and Figure 2), a validated RA risk locus among Europeans and Asians (10,47,48). We found that rs11681966 was suggestively associated with RA in African Americans (OR = 1.5, 95% CI 1.23–1.78, $p = 4.04 \times 10^{-5}$). The lead *AFF3* SNP (rs9653442) associated with RA in European ancestry ($p = 3.6 \times 10^{-12}$) (10) was not strongly associated with RA susceptibility ($p = 0.015$) in African Americans. Similarly, rs10209110, the index variant in *AFF3* in another study of RA in Europeans (4), was not associated in our dataset ($p = 0.84$). Therefore, due to differing association strengths and LD patterns in the locus, we conducted trans-ethnic fine mapping of this locus using data from African Americans, Asians and European RA patients and controls using PAINTOR3 (39) (see Methods).

Most association studies on *AFF3* have examined roughly the region from chr2:100,800,000 to 100,850,000, which contains index variants identified by multiple prior GWAS. However, our index variant (rs11681966, at chr2:100,759,457) is outside this region, located near the 5′ end of *AFF3* (>1 kb). Thus, we defined the risk locus as a broader region (from chr2:100,709,000 to 100,875,000). We then conducted trans-ethnic fine mapping using PAINTOR 3. Figure 2 shows the 90% credible set for variants in the *AFF3* locus, enriched genomic annotations used to help construct the credible set and zoom plots in European, Asian and African American populations with LD heatmaps for each (Figures 2B–D, respectively). Doing so revealed that rs11681966 and a linked variant, rs13003982, were also in the 90% credible set defined by PAINTOR3 (see Figure 2A and Table 3). Consistent with previous reports of autoimmune disease in other ethnicities, our trans-ethnic fine mapping analysis of the *AFF3* locus in combined African American, European and Asian RA identified rs9653442, rs6712515 and rs7608424 as likely candidates to be pathogenic variants (see Figure 2 and Table 3). Several of these SNPs are listed as index variants in the National Human Genome Research Institute (NHGRI) GWAS catalog (49) and have been noted in prior studies (50).

Association between iChip Markers and Radiographic Severity of RA

After quality control procedures, 100,169 SNPs with MAF >0.05 were available for analysis in 548 autoantibody-positive RA patients who had radiographic scores. A Manhattan plot illustrating the genetic variants associated with severity is shown in Figure 3. In contrast to studies in individuals of European ancestry, we did not find a statistically significant association between SNPs tagging the *HLA* region and radiographic severity (Table 4). We detected several suggestive associations, including variants in or near *AFF3* (Supplementary Figure S3A), *TNFRSF9* (Supplementary Figure S3B), *CTLA4* (Supplementary Figure S3C), *IL2RA* (Supplementary Figure S3D), *C5/TRAF1* (Supplementary Figure S3E) and *NALCN/ITGBL1* (Supplementary Figure S3F). rs506746 (near *NALCN/ITGBL1*) was the most strongly associated variant with radiographic severity ($p = 4.33 \times 10^{-7}$), but we could not evaluate support from LD for this association due to low marker density for this region on the iChip array, so no further analysis was performed.

We chose to examine two loci (*IL2RA* and *ETS1*) in more detail. Multiple *IL2RA* variants have been associated with autoimmune conditions (juvenile idiopathic arthritis, type 1 diabetes, systemic lupus erythematosus [SLE], multiple sclerosis, Graves' disease and so on) (49). Similar to previous reports, we observed a suggestive association in the *IL2RA* locus (rs7077067) with radiographic severity ($p = 5.16 \times 10^{-5}$) (51). rs7077067 was the lead SNP in this study, which differs from that in Europeans, rs2104286 (51). In our study, rs2104286 had MAF = 0.05 and was only weakly associated with RA

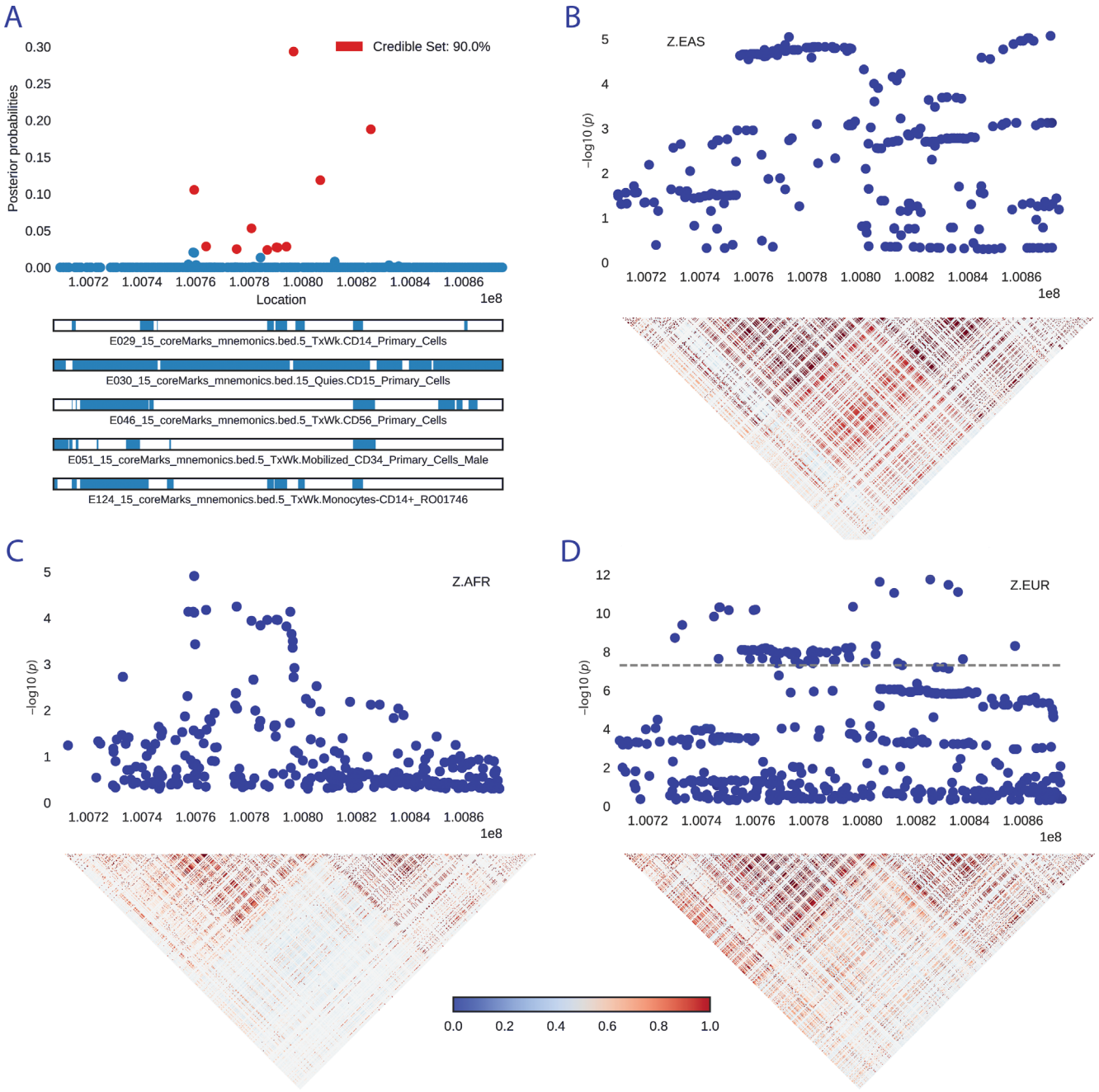

Figure 2. Results from trans-ethnic fine mapping of the *AFF3* locus. (A) The 90% credible set for candidate pathogenic variants (top) and selected annotations used to prioritize variants, with blue coloration indicating variants having a given annotation (bottom). (B–D) Zoom plot of association summary statistics versus genomic position for Asians, African Americans, and Europeans with RA. Each includes a heatmap for variants in the locus, colored according to a linkage disequilibrium LD heatmap generated from that population. The color bar (bottom middle) indicates the degree of linkage disequilibrium for variants in each LD heatmap.

radiographic severity ($p = 0.024$). There is a paucity of trans-ethnic association summary statistics for RA radiographic severity. Thus, we relied on conditional analysis, pairwise LD estimates and prior studies (not trans-ethnic fine mapping) to further understand these loci. Adjusting for the effect of rs2104286 did not eliminate the association of rs7077067 with severity ($p = 8.15 \times 10^{-5}$). Previous studies in other ancestries have noted substantial LD between variants in *IL2RA* and the

Table 3. Ninety percent credible set for pathogenic variants in trans-ethnic fine mapping of *AFF3* locus in RA susceptibility.

rsID	Chr	Position	Effect allele	Alternate allele	Z AA[a]	Z EAS[b]	Z EUR[c]	Posterior probability
rs13003982	chr2	100759078	T	C	–3.80	–4.09	–5.65	0.021
rs11681966[d]	chr2	100759457	A	C	–4.22	–4.09	–5.65	0.105
rs12712067	chr2	100763900	T	G	–3.82	–4.08	–5.69	0.029
rs4851257	chr2	100775297	T	C	–3.86	–4.14	–5.59	0.025
rs4851258	chr2	100780830	T	C	–3.68	–4.17	–5.60	0.053
rs4851261	chr2	100786717	A	G	–3.69	–4.17	–5.60	0.024
rs10185059	chr2	100790172	T	C	–3.70	–4.16	–5.63	0.027
rs10185510	chr2	100790581	T	C	–3.69	–4.16	–5.63	0.027
rs12712071	chr2	100793876	A	G	–3.61	–4.17	–5.69	0.028
rs7608424[d]	chr2	100796543	T	G	–2.89	–4.15	–6.48	0.293
rs6712515[d]	chr2	100806514	T	C	–2.31	–3.66	–6.91	0.119
rs9653442[d]	chr2	100825367	T	C	–2.43	–3.50	–6.95	0.188

Chr: chromosome.
[a]Z-score from our study of African Americans.
[b]Z-score for East Asians from the trans-ethnic meta-analysis of Okada *et al.*
[c]Z-score for Europeans from Okada *et al.*
[d]Denotes that the variant was the index variant reported in this study, or in another genome-wide association study.

surrounding region, including *RBM17* (51,52). In this locus, we found shorter haplotype blocks and lower LD between genetic variants, so we sought to localize an association signal in this locus. We found that the most strongly associated SNPs in our dataset are in the first intron of *RBM17*, specifically in a ~5kb section of the genome displaying the H3K27Ac histone marks and DNAse hypersensitivity (Figure 4).

With regard to *ETS1*, we found that rs4362159 was associated with radiographic severity ($p = 6.26 \times 10^{-5}$). We also identified a variant linked to rs4362159, rs7108537, which was more weakly associated with RA radiographic severity ($p = 2.8 \times 10^{-4}$), but exists in the transcription factor binding site–rich region. Similarly, a previous study of SLE identified rs6590330 as an SLE risk variant that alters binding of pSTAT1 and affects *ETS1* expression in persons of Asian ancestry only. As expected, this SNP was not associated with RA radiographic severity ($p = 0.11$) in our dataset, nor was the lead SNP in our study in LD with rs6590330 ($r^2 = 0.03$), or with other previously described variants reported in the NHGRI GWAS catalog (49), for example, rs1128334 ($p = 0.27$; $r^2 = 0.01$) (53).

DISCUSSION

Our analyses led to several important findings regarding RA in African Americans. First, SNPs tagging *HLA-DRB1* were significantly associated with RA susceptibility, but not radiographic severity. Second, *AFF3*, *TNFSF11* and *TNFSF18* (all previously validated loci for RA susceptibility) were associated suggestively with RA susceptibility ($1.0 \times 10^{-4} < p < 3.1 \times 10^{-6}$). Third, *TNFRSF9*, *CTLA4*, *IL2RA*, *C5/TRAF1* and *CXCR5* were associated suggestively with radiographic severity. Finally, leveraging the differential LD pattern between Europeans and African Americans, we defined suggestive novel lead SNPs for the associations of *AFF3* with susceptibility and *IL2RA* with severity.

As expected from previous studies and our prior work (18), we found that the strongest association with RA susceptibility lies in the MHC region near *HLA-DRB1*. When the SNPs in the extended

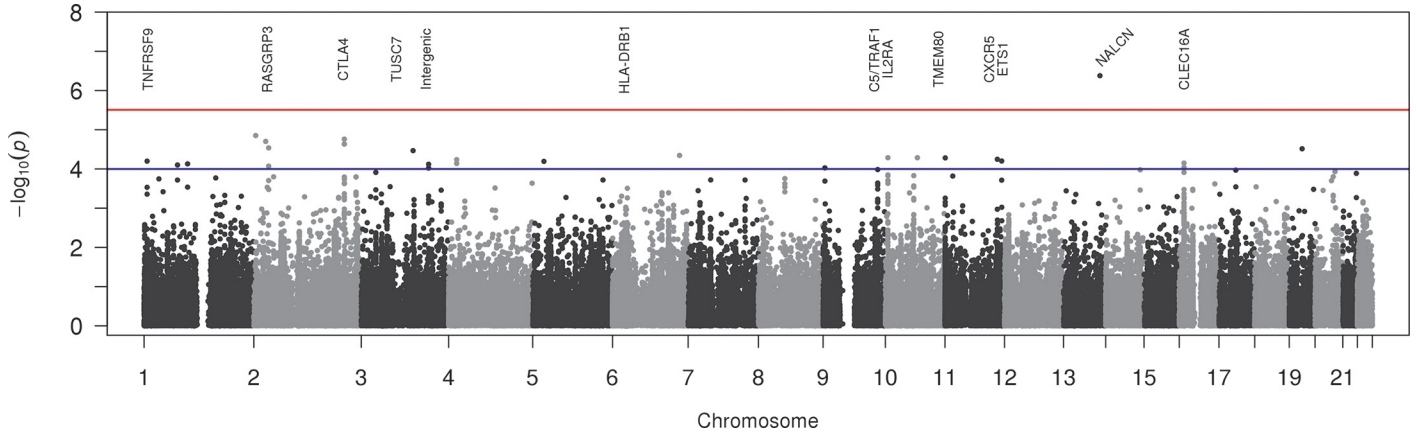

Figure 3. Manhattan plot of the association of iChip variants with radiographic severity in African Americans with autoantibody-positive RA. The x-axis indicates chromosome and position, the y-axis indicates association strength –log(p). The blue line illustrates suggestive statistical association ($p = 1*10^{-4}$). The red line illustrates the iChip-wide level of statistical significance ($p = 3.1*10^{-6}$).

Table 4. Variants genotyped on the iChip associated with RA radiographic severity in African Americans. Zero and count refer to the model coefficients for the portions of the zero inflated negative binomial model.

rsID	Chr	Position	A1[a]	Statistic	Effect size	95% CI	*P* value	Gene locus
rs228702	1	7945520	G	IRR	0.58	0.44–0.76	6.33×10^{-5}	*TNFRSF9*[b]
rs13014054	2	33678924	A	IRR	0.53	0.39–0.71	2.92×10^{-5}	*RASGRP3*
rs73055463	2	204712807	C	OR	1.99	1.38–2.86	4.72×10^{-5}	*CTLA4*[b]
rs7034499	9	123687231	C	OR	2.27	1.49–3.46	9.60×10^{-5}	*TRAF1-C5*[b]
rs7077067	10	6132692	A	IRR	1.48	1.22–1.78	5.16×10^{-5}	*IL2RA*[b]
rs7101785	11	696437	G	IRR	1.52	1.24–1.86	5.17×10^{-5}	*TMEM80*
rs7127742	11	118521637	G	IRR	0.40	0.25–0.62	5.66×10^{-5}	*PHLDB1/CXCR5*
rs4362159	11	128305571	A	IRR	0.48	0.34–0.69	6.26×10^{-5}	*ETS1*[b]
rs506746	13	101981771	A	IRR	0.53	0.41–0.68	4.19×10^{-7}	*NALCN/ITGBL1*
rs7193451	16	11050356	G	IRR	1.65	1.30–2.09	7.09×10^{-5}	*CLEC16A*

Chr: chromosome; IRR: incident rate ratio.
[a]The allele tested in this study.
[b]Indicates a validated risk locus for RA.

MHC were conditioned on the classical *HLA-DRB1* alleles, the association signal elsewhere in the MHC region is lost. This finding illustrates that the genome-wide significant SNPs in our study are tagging *HLA-DRB1* classical alleles. This was first noted in Europeans (46), but in that study residual significant association signal remained near *HLA-B* and HLA-*DPB1* after the conditioning analysis. We observed residual signals near *HLA-B* and *HLA-DPB1* having the same effect size and direction of effect, but they were not significantly associated with RA. Considering the consistency of effect size, this likely reflects statistical power, but could reflect biological differences as well.

Despite our study being well powered to detect an effect of similar magnitude, we found no association between MHC region SNPs and radiographic severity comparable to other reports. Viatte *et al.* reported an association between haplotypes defined by amino acid residues at positions 11, 71 and 74 of *HLA-DRB1* and radiographic damage (15). There are several possible explanations for this discrepancy. First, the differences could result from cohort inclusion criteria. Viatte *et al.* included autoantibody-positive RA, autoantibody-negative RA and inflammatory polyarthritis (not meeting ACR criteria for classification of RA), while our study focused exclusively on autoantibody-positive RA. Second, because they did not stratify based on autoantibody positivity, it is possible that their findings reflect the known association between radiographic severity and autoantibody positivity. Finally, biological differences between ethnicities cannot be ruled out.

AFF3 encodes LAF4, a transcriptional activator with suspected roles in lymphoid tissue development and oncogenesis (54). The locus has been associated with RA susceptibility in Europeans (4,10) as well as SLE and juvenile idiopathic arthritis (10,55,56). rs9653442 in particular has been the subject of several investigations as an autoimmune risk variant, and it was the index variant for RA risk in a trans-ethnic meta-analysis (10). In this study, it was found in the 90% credible set for pathogenic variants. However, the *AFF3* locus has been identified as containing multiple independent effects for common complex diseases (50). Consistent with this, our results further suggest several promising candidate pathogenic variants in addition to rs9653442. rs6712515 is an index variant reported in the NHGRI GWAS catalog (49), but has previously been associated with cognitive phenotypes rather than autoimmunity. These two variants as well as rs7608424 are known to be expression quantitative trait loci for *AFF3* expression (57). The index variant in our study, rs11681966, is found only ~400 bases from the transcription start site of *AFF3* in a conserved region capable of binding numerous transcription factors. Another variant in tight linkage with rs11681966, rs13003982, is located only ~40 bp from the transcription start site of *AFF3*. Thus, our data not only suggest candidate pathogenic variants, but an initial finding for functional studies of the contribution of RA genetic variants to *AFF3* to test.

We also detected several suggestive associations with RA radiographic severity. *CTLA4* is associated with RA in several populations (10,58), and the importance of CD28/CTLA4 co-stimulation in RA is highlighted by the efficacy of CTLA4Ig (abatacept) (59). We detected a suggestive association of *TNFRSF9* with RA radiographic severity. *TNFRSF9* (CD137) is a member of the TNF receptor family known for its role in T cell co-stimulation. In RA, a soluble form of CD137 is released by activated lymphocytes and is present in the serum (60). In collagen-induced arthritic mice, treatment with an anti-CD137 antibody protects against disease progression, possibly by amplifying antigen-specific CD11c + /CD8 + T lymphocytes and suppressing the pathogenic CD4 + T lymphocyte subset (61). While rs506746 (chr13:101981771, near *NALCN* and *ITGBL1*) showed the strongest association with radiographic severity, this finding should be interpreted cautiously because of the low coverage of this region on the iChip array.

We subjected two loci to additional analyses based on context provided by prior studies. We observed a suggestive association between RA severity and rs7077067, a variant near *IL2RA*. This locus has previously been linked to RA susceptibility (10), radiographic severity (31,51) and decreased likelihood of disease remission (62). Interleukin 2 receptor α (*IL2RA* or *CD25*) gene, together

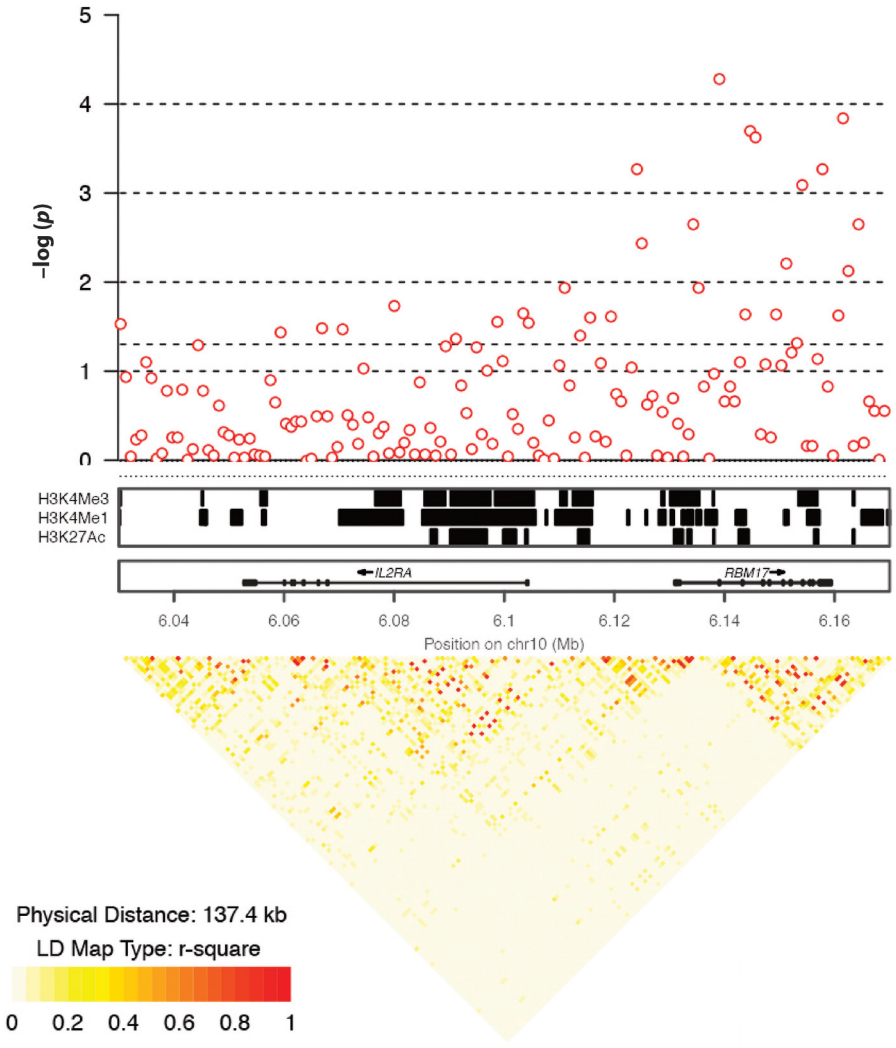

Figure 4. (Top) Zoom plot indicating the strength of association in the region of chromosome 10 surrounding the *IL2RA* locus. Circles represent single nucleotide polymorphisms (SNPs); the y-axis measures negative log association *p* value and the x-axis represents genomic position. (Middle) Diagram indicating histone markings and gene diagrams corresponding to the genomic region in the zoom plot. (Bottom) LD heatmap for the region.

with *IL2RB* and *IL2RG*, encodes the high-affinity IL2 receptor. In the absence of *IL2RA*, there is abnormal proliferation and migration of T cells, resulting in widespread inflammation. This may be due to reduced T cell apoptosis in the thymus, resulting in autoreactive T cell survival (63). In addition, rs2104286 in the *IL2RA* locus has been shown to reduce T cell activation in healthy individuals (64) and is associated with radiographic severity of RA in Europeans (49,52). Specifically, the minor allele of rs2104286 was associated with decreased progression of joint destruction and lower levels of soluble IL-2Rα. rs7077067 was not in LD ($r^2 \leq 0.02$) with any of the 10 index variants previously reported, including rs2104286. The relatively weak association of rs2104286 in our study and the low LD suggest that additional pathogenic variants may be found upstream of *IL2RA*. It is possible that different risk haplotypes predominate in African Americans with RA. Alternatively, low LD between variants in the *IL2RA* locus may preclude tagging the same pathogenic variant.

We also found an association of radiographic severity of RA with *ETS1*, a highly conserved transcription factor whose expression in B cells, T cells and natural killer cells strongly affects immune cell function. *Ets1* knockout mice display aberrant T cell differentiation, altered cytokine expression and increased differentiation into memory and effector T cells (65). Downregulation of Ets1 increases formation of plasma cells in part by upregulating Pax-5 and inhibiting Blimp1 activity (66). In humans, lupus risk alleles are associated with lower *ETS1* mRNA expression, and the genetic basis of these findings differs in an ethnic-specific fashion (67). Specifically, increased binding of pSTAT1 to oligonucleotides containing the rs6590330 risk allele correlates with decreased *ETS1* expression in Asian SLE patients, but not in other populations, including African Americans (67). Consistent with this finding, we found that rs7108537, but not rs6590330, was associated with radiographic severity. There are transcription factor binding sites in the immediate vicinity of rs7108537, and the genotype of this SNP appears to affect *ETS1* expression in persons of Yoruban ancestry, but not in other populations (68). Therefore, our study of radiographic severity provides additional evidence that population-specific variants may contribute to risk of autoimmunity by decreasing *ETS1* expression.

Our association testing results should be interpreted cautiously, as the sample size may result in inflated effect size estimates. In addition, our study was not well powered to detect association of common SNPs (MAF 0.15-0.50) with effect sizes <1.3. Nevertheless, we used the largest registry of African Americans with RA for whom clinical, radiographic and genetic data are available. We were unable to attempt to replicate our findings, because no other cohorts of African Americans with RA are available. Finally, it should be noted that the markers

selected during the design of the custom iChip array were derived from the 1000 Genomes project from European individuals, which might be suboptimal for analysis of disease-associated variants in African Americans, and thus additional novel risk variants RA may yet exist in this ethnic group. Limitations of the fine mapping study include exclusion of some genotyped variants due to absence from the reference dataset used for LD and inability to confirm uniform alignment of some variants to reference genomes based on LD and Z-score information. This may lead to the exclusion of potentially interesting variants. For instance, rs11676922, an RA index SNP previously studied in a meta-analysis of RA in Han Chinese and Europeans (69), was not examined in this study. This SNP is in near-perfect LD with rs9653442 as well as rs6712515. As such, investigators who wish to carry out functional studies on variants in *AFF3* should note that including this variant might alter the posterior probabilities attributed to other variants.

CONCLUSION

In contrast to other reports, we find that SNPs in the MHC region do not appear to be associated with radiographic severity of RA in African Americans. Our study also demonstrates the utility of ethnic-specific analysis of genetic data. We confirm the association of *AFF3* with RA susceptibility and *IL2RA* and *ETS1* with radiographic severity, and our analysis of these loci suggests several candidate variants for functional validation. Our analysis of the *AFF3* locus suggests that rs11681966, rs9653442, rs7608424 and rs6712515 are high-priority targets for functional studies, but may exert effects in population-specific contexts. Our data add to evidence that *ETS1* autoimmune risk is mediated by ethnic-specific variants decreasing expression. In the *IL2RA* locus, our data suggest that trans-ethnic fine mapping studies could be valuable for RA susceptibility and radiographic severity due to different LD patterns. Overall, our study suggests that trans-ethnic genetic analysis is likely to be an important step in bringing precision medicine to complex autoimmune diseases, including RA.

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DISCLOSURES

The authors declare they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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