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## The HLA–DRB1 Shared Epitope Is Associated With Susceptibility to Rheumatoid Arthritis in African Americans Through European Genetic Admixture

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### Abstract

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#### AUTHOR CONTRIBUTIONS

Dr. Bridges had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study design.** Jonas, Smith, Howard, Moreland, Bridges.

**Acquisition of data.** Hughes, Westfall, Dwivedi, Holers, Conn, Jonas, Callahan, Smith, Gilkeson, Moreland, Patterson, Reich, Bridges.

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**Objective**—To determine whether shared epitope (SE)-containing HLA-DRB1 alleles are associated with rheumatoid arthritis (RA) in African Americans and whether their presence is associated with higher degrees of global (genome-wide) genetic admixture from the European population.

**Methods**—In this multicenter cohort study, African Americans with early RA and matched control subjects were analyzed. In addition to measurement of serum anti-cyclic citrullinated peptide (anti-CCP) antibodies and HLA-DRB1 genotyping, a panel of >1,200 ancestry-informative markers was analyzed in patients with RA and control subjects, to estimate the proportion of European ancestry.

**Results**—The frequency of SE-containing HLA-DRB1 alleles was 25.2% in African American patients with RA versus 13.6% in control subjects ( $P = 0.00005$ ). Of 321 patients with RA, 42.1% had at least 1 SE-containing allele, compared with 25.3% of 166 control subjects ( $P = 0.0004$ ). The mean estimated percent European ancestry was associated with SE-containing HLA-DRB1 alleles in African Americans, regardless of disease status (RA or control). As reported in RA patients of European ancestry, there was a significant association of the SE with the presence of the anti-CCP antibody: 86 (48.9%) of 176 patients with anti-CCP antibody-positive RA had at least 1 SE allele, compared with 36 (32.7%) of 110 patients with anti-CCP antibody-negative RA ( $P = 0.01$ , by chi-square test).

**Conclusion**—HLA-DRB1 alleles containing the SE are strongly associated with susceptibility to RA in African Americans. The absolute contribution is less than that reported in RA among populations of European ancestry, in which ~50–70% of patients have at least 1 SE allele. As in Europeans with RA, the SE association was strongest in the subset of African American patients with anti-CCP antibodies. The finding of a higher degree of European ancestry among African Americans with SE alleles suggests that a genetic risk factor for RA was introduced into the African American population through admixture, thus making these individuals more susceptible to subsequent environmental or unknown factors that trigger the disease.

Rheumatoid arthritis (RA) is characterized by inflammation in the synovial membrane of diarthrodial joints. The cause of RA is unknown, but both environmental factors and genetic susceptibility appear to be involved. Although RA is consistently shown to have a prevalence of ~1% among populations of European ancestry (1), there appears to be a relatively low prevalence among black Africans, particularly those living in rural settings, and its prevalence in African Americans is not well described. The reported prevalence of RA in rural regions of Africa has ranged from 0% to 0.68% of the populations under study (2–7).

The HLA encoding the major histocompatibility complex (MHC) is the genetic region with the strongest association with RA in persons of European ancestry (8). The HLA-DRB1 alleles associated with RA (\*0401, \*0404, \*0405, \*0408, \*0413, \*0101, \*0102, \*1402, and \*1001) encode a common sequence at amino acids 70–75 (QKRAA) in the third hypervariable region of the  $\beta$ -chain, referred to as the shared epitope (SE) (9–11). HLA-DRB1 alleles containing the SE are found in ~50–70% of RA patients of European ancestry (1,12). Association of RA with HLA-DRB1 alleles is observed in all racial/ethnic populations studied to date, but there is a paucity of data on Africans and African Americans. The particular SE-containing alleles appear to vary by racial/ethnic group. HLA-DRB1\*0401 and \*0404 are most common in Northern Europeans and persons of European ancestry living in the US (13), while \*1402 appears to be important in Native Americans (14). HLA-DRB1\*0101 and \*1001 have been reported among Israelis, Greeks, and Spaniards with RA (15–17). Among Asian populations (Koreans, Japanese, and Chinese), HLA-DRB1\*0405 may be the dominant RA-associated allele (18–20). HLA-DRB\*09 alleles have also been reported to influence susceptibility to RA in Koreans,

Chileans, and Japanese, as well as persons of European ancestry living in the UK (18,21–23).

According to the 1995 National Marrow Donor Program (comprising more than 1.35 million HLA-typed healthy volunteers), there are significant differences in frequencies of HLA–DR4 alleles (as defined by micro-lymphocytotoxicity serologic assays) between persons of European ancestry (mean  $\pm$  SEM gene frequency  $16.8 \pm 0.05\%$ ) and African Americans (mean  $\pm$  SEM gene frequency  $5.7 \pm 0.08\%$ ) (24). Characterization of HLA–DRB1 alleles from 564 consecutively recruited African American volunteers for a hematopoietic stem cell registry was recently reported (25). In a study by Silman et al, HLA–DRB1\*04 alleles were observed in only 1 (1.8%) of 55 persons in a rural Nigerian population (6).

Because few studies have focused on African Americans with RA, the role of HLA–DRB1 alleles in that ethnic group is unclear. McDaniel et al (26) observed that alleles \*0401 to \*0411 represented 18 (14.8%) of 121 alleles in 66 African American patients with rheumatoid factor (RF)–positive RA, 5 (14.7%) of 34 alleles in 20 patients with RF-negative RA, and 17 (6.9%) of 247 alleles in 130 control subjects. Of 57 African American patients in the Minocycline in RA trial, approximately one-third had at least 1 SE allele (27). Del Rincón and Escalante (28) analyzed HLA–DRB1 genotypes of RA patients of different ethnic groups, including 53 African Americans. They observed that \*04 alleles were significantly less common among African American patients with RA than among non-Hispanic white patients (odds ratio [OR] 0.19, 95% confidence interval [95% CI] 0.09–0.41). HLA–DRB1\*09, \*12, \*13, and \*16 were enriched in African Americans with RA compared with non-Hispanic whites. Given these data, we sought to determine the degree to which HLA–DRB1 alleles associated with RA in persons of European ancestry are associated with RA in African Americans.

Genetic admixture between European and African populations, which was brought to the Americas as part of the slave trade, might be a contributing factor to the possible difference in the prevalence of RA in African Americans compared with Africans. Although the majority of slaves originated from western Africa, a substantial number were from central Africa as well. African Americans have, on average, 20% European ancestry, resulting from admixture that has occurred largely within the past 15 generations (29). Because of the limited number of generations of admixture, there are extended stretches of DNA containing contiguous European and African ancestry (29). Estimates of European ancestry can be made by genotyping sets of ancestry-informative markers (AIMs; ethnic difference markers) (29), defined as single-nucleotide polymorphisms, in which there are much higher minor allele frequencies in one ethnic group compared with the other. Given the differences in frequencies of HLA–DRB1 alleles containing the SE in Africans versus persons of European ancestry, we hypothesized that if there was an association between these alleles and RA in African Americans, there would be selective enrichment of European ancestry among those individuals with the HLA–DRB1 SE.

The most prominent autoantibody in RA is RF, which is directed against the Fc portion of IgG, but antibodies directed against citrullinated peptides have been found to be highly specific for RA (for review, see ref. 30). We recently reported the diagnostic utility of anti-cyclic citrullinated peptide (anti-CCP) antibodies in African Americans with early RA, using data and sera from the subjects in the Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis (CLEAR) registry (31). It has recently been appreciated that in persons of European ancestry, the HLA–DRB1 SE is associated with anti-CCP-positive RA and not with anti-CCP-negative RA (32). The association of HLA–DRB1 alleles and the presence of anti-CCP antibodies has not been examined in African Americans with RA. The final hypothesis we sought to test was that HLA–DRB1 alleles

encoding the SE were associated with anti-CCP antibody–positive RA, but not anti-CCP antibody–negative RA, in African Americans.

In summary, the goals of this study were 1) to determine whether RA in African Americans is associated with the HLA–DRB1 SE, 2) to determine whether the presence of the SE is attributable to a higher degree of global (genome-wide) European admixture, and 3) to determine whether the HLA–DRB1 SE is associated with the subset of anti-CCP antibody–positive RA in African Americans.

## PATIENTS AND METHODS

### Patients and control subjects

The CLEAR registry is a National Institute of Arthritis and Musculoskeletal and Skin Diseases–funded registry enrolling self-identified African Americans with early RA (disease duration  $\geq 2$  years) from 4 sites: the University of Alabama at Birmingham (Coordinating Center), Emory University/Grady Hospital, Atlanta, Georgia, the University of North Carolina at Chapel Hill, and the Medical University of South Carolina at Charleston. (For a list of the CLEAR investigators, see Appendix A.) Comprehensive demographic, clinical, and radiographic data are being collected on CLEAR participants, and serum and DNA samples are being stored. At present, baseline demographic and medical data, as well as DNA and serum samples, are available on African American patients with RA and healthy African American control subjects matched for geographic location. The CLEAR registry is a national resource, with clinical data, DNA, and other biologic samples available (for details, see the following Web site: <http://www.dom.uab.edu/rheum/CLEAR%20home.htm>). All study procedures were compliant with the American Health Insurance Portability and Accountability Act of 1996 and were approved by the University of Alabama Institutional Review Board for Human Use.

Subjects available for analysis in this case–control study included the initial 325 patients with RA enrolled in the CLEAR registry and 116 African American control subjects matched for age, sex, and geographic location (see below). In addition, DNA samples from 50 healthy African American work site volunteers from the Birmingham area were kindly provided by Drs. J. Edberg and R. Kimberly. The latter 2 samples constitute the total control group of 166 individuals. Baseline characteristics of the CLEAR patients are shown in Table 1.

CLEAR control subjects were recruited predominantly based on lists of telephone numbers for individuals with the same zip codes as those of the patients. These lists were obtained from Genesys/Marketing Systems Group (online at <http://www.m-s-g.com/default.htm>). Telephone numbers were selected from census tracts with high percentages of African Americans living near the sites enrolling CLEAR patients. Control subjects were selected from within an age range ( $\pm 10$  years) based on the mean age of patients at the sites, with a female:male ratio of 3:1 to match that of the RA patient group. Potential control subjects were called by interviewers to determine eligibility and interest, and lists of suitable control subjects were then distributed to the sites to arrange study visits.

### HLA–DRB1 genotyping

High-resolution HLA–DRB1 genotyping was determined on 321 of the 325 patients with RA and 164 of the 166 control subjects by DNA sequencing of exon 2, using the AlleleSEQR HLA–DRB1 reagent kit and protocol (Atria Genetics, South San Francisco, CA). After polymerase chain reaction amplification of HLA–DRB1 exon 2 from genomic DNA, forward and reverse cycle sequencing was performed, and the resulting fragments were collected and analyzed on an ABI 377 automated sequencer (Applied Bio-systems,

Foster City, CA). An additional sequence reaction was performed to analyze the GTG (valine) motif of codon 86 sequences, thus enabling resolution of ambiguous results for some exon 2 sequences. The sequences were analyzed using Assign software (Conexio Genomics, Fremantle, Western Australia, Australia), which enables assignment of genotypes based on a recent library file of HLA–DRB1 alleles. This method detects all of the SE-positive alleles listed above. When needed, group-specific amplifications were performed and sequence analysis carried out in order to differentiate among alleles within a particular group.

### Admixture genotyping and analysis

DNA samples from 325 CLEAR patients with RA were genotyped at the Broad Institute of Massachusetts Institute of Technology and Harvard, using the BeadLab platform (Illumina, San Diego, CA) on a set of 1,312 AIMs chosen to be extremely different in frequency between West Africans and European Americans (panel 1) (29,33), yielding estimates of the proportion of genome-wide European ancestry for each individual. Genotyping was subsequently performed on 85 CLEAR control subjects, using an updated panel of 1,530 AIMs (panel 2). Markers in these panels of AIMs were distributed throughout the genome, so the estimates reflect global admixture. A total of 596 AIMs were common to both panel 1 and panel 2. Data filtering and cleaning procedures were identical to those reported in a recent study on admixture mapping of multiple sclerosis genes (33).

The percentage of subjects of European ancestry was calculated based on the genotypes of the AIMs, using the software package AdmixMap, version 3.1 (ref. 34 and online at <http://www.ucd.ie/genepi/software.html>). AdmixMap utilizes data from the founding populations to provide informative prior distributions of allele frequencies that are used to calculate the posterior distribution of individual admixture estimates, using the Markov chain Monte Carlo method in a Bayesian framework (35,36).

As mentioned above, 2 overlapping but different panels of AIMs were used to calculate the percent European ancestry for patients with RA (panel 1) and control subjects (panel 2). To look for differences in the estimated percent admixture between patients with RA and control subjects attributable to the use of different panels of AIMs, we recalculated the percent European admixture for patients with RA and control subjects based solely on the 596 AIMs present in both panel 1 (patients) and panel 2 (controls). Admixture-based mapping of RA susceptibility alleles was conducted with AncestryMap software, as previously described (33,37).

### Measurement of anti-CCP antibodies

Anti-CCP (IgG) antibodies were measured, as previously described, in CLEAR patients with RA and control subjects (31). Commercially available second-generation (anti-CCP-2) enzyme-linked immunosorbent assay kits (Diastat; Axis-Shield Diagnostics, Dundee, Scotland, UK) were used, and the assays were performed according to the manufacturer's instructions. Anti-CCP antibodies were measured in arbitrary units per milliliter and were considered to be positive at a cutoff value  $\geq 5$  units/ml, which was determined using serum collected from a control population.

### Statistical analysis

The frequencies of individual HLA–DRB1 alleles were compared between patients with RA and control subjects, using chi-square or Fisher's exact testing, as appropriate. Similarly, the presence or absence and the number of alleles bearing the SE (0 versus 1 versus 2) were compared between patients with RA and control subjects and between patients with anti-CCP antibody-positive RA and those with anti-CCP antibody-negative RA.

For subjects for whom complete admixture and HLA–DRB1 data were available, we analyzed potential associations between the estimated proportion of European ancestry and the HLA–DRB1 SE, both its presence versus absence and number of alleles containing the SE (0, 1, or 2), using a Spearman’s rank order coefficient. We compared patients with control subjects and also analyzed association between admixture and SE status, considering patients and control subjects as one group, independent of disease status.

In addition, logistic regression models were used to analyze the odds of having an SE allele as a function of European ancestry. One model was used for each HLA–DRB1 allele containing the SE. We also used additive genetic effect (linear) models to detect differences in admixture between patients and control subjects and between subjects with 0, 1, or 2 SE-containing HLA–DRB1 alleles.

In order to detect potential spurious results from differences in panels 1 and 2, the models used AdmixMap results separately for patients (AIM panel 1) and control subjects (AIM panel 2), as well as for the 596 AIMs common to both panel 1 and panel 2. Each of these AIMs was fitted using risk alleles with 3 categories (0, 1, or 2 SE alleles) or 2 categories (SE present versus absent). The models were then extended by adding the group factor (patient or control), first testing for the interaction and then dropping it if not significant.

## RESULTS

### Association of HLA–DRB1 alleles with RA in African Americans

Table 2 displays the distribution of HLA–DRB1 alleles in African Americans with early RA and control subjects. The frequency of HLA–DRB1 alleles containing the SE was 25.2% (162 of 642) in African American RA patients versus 13.6% (45 of 332) in African American control subjects (OR 2.12, 95% CI 1.46–3.10,  $P=0.00005$ , by chi-square test). Of the 321 patients with RA, 135 (42.1%) had at least 1 allele containing the SE (111 with 1 SE allele, 24 with 2 SE alleles). In contrast, only 42 of 166 control subjects (25.3%) had at least 1 allele containing the SE (38 with 1 SE allele, 4 with 2 SE alleles) (OR 3.94, 95% CI 1.39–3.31,  $P=0.0004$ , by chi-square test) (Figure 1). There were significant differences in the frequencies of particular alleles. In African American patients with RA, the frequency of the \*0401 allele was 5.6% (36 of 642 alleles); in African American controls the frequency was 1.2% (4 of 332 alleles) ( $P=0.0004$ , by Fisher’s exact test). The \*0404, \*0405, and \*1001 alleles were also significantly more frequent among patients than controls (see Table 2 for  $P$  values). In contrast, the control group had a higher frequency of the \*1101 allele (44 [13.3%] of 332 alleles) than the RA patient group (35 [5.5%] of 642 alleles;  $P=0.00004$ , by chi-square test), which might lead to speculation that it is a “protective” allele. The differences between patients with RA and control subjects were confirmed when patients with RA were compared with healthy African American volunteers from a hematopoietic stem cell registry (25) (see Table 2).

Because our control group consisted of 2 subsets, we compared HLA–DRB1 genotypes and allele frequencies between CLEAR patients with RA and each of the 2 individual subsets of our control group. As shown in Table 3, there were statistically significant differences between patients and both sets of controls, which became stronger when the controls were analyzed as a whole. In addition, the distribution of HLA–DRB1 alleles in our controls was very similar to that reported in 564 healthy African Americans (25).

### Association of the HLA–DRB1 SE with a higher degree of European admixture in patients with RA and control subjects

Admixture and HLA–DRB1 genotype data were available for 313 patients with RA and 82 control subjects. Figure 2 shows the distributions of European admixture for patients and

control subjects. The estimated percent European ancestry in the CLEAR RA patients ranged from 0.9% to 54.0% (mean  $\pm$  SEM 16.0  $\pm$  2.3%). Among control subjects, the range was 3.1–36.0% (mean  $\pm$  SEM 14.8  $\pm$  2.3%) (Figure 2). Table 4 displays the mean percent European ancestry in patients with RA and control subjects stratified by HLA–DRB1 SE status. Table 5 displays the results of additive genetic effects models and Spearman’s rank order coefficients of association between the percent European ancestry and SE status. There was a significant association between the percent European ancestry and SE status when RA patients and controls were combined. The association appeared stronger when restricting the analysis to the 596 AIMs common to panels 1 and 2. For example, among the combined patients with RA and control subjects, the percent European ancestry was strongly associated with number of SE alleles ( $P=0.02$  for both the additive genetic effects model and the Spearman’s rank order coefficient). The association appeared stronger when restricting the analysis to the 596 AIMs common to panels 1 and 2 ( $P=0.02$  and  $P=0.005$  for the additive genetic effects model and Spearman’s rank order coefficient, respectively). These findings indicate that a higher percent European admixture is associated with a higher likelihood of having the HLA–DRB1 SE.

Table 6 shows the results of the additive genetic effects models for European admixture with the SE and disease status (RA versus control). RA status was not significantly related to the percent European ancestry, whereas the SE was associated with higher degrees of European ancestry. For example, the percent European admixture was associated with the number of SE alleles ( $P=0.02$  for panels 1 and 2) but not with RA ( $P=0.55$ ).

Logistic regression models were used to determine the association between each of the specific alleles encoding the SE and the percent European ancestry. HLA–DRB1\*0401, but no other individual HLA–DRB1 SE allele, was significantly related to European ancestry (OR 1.035, 95% CI 1.004–1.068,  $P=0.028$ ). Specifically, for every 1% increase in European ancestry, the odds of having the \*0401 allele increased by a factor of 1.035. There was marginal association between the percent European ancestry and the presence or absence of the SE (OR 1.02, 95% CI 0.997–1.004,  $P=0.095$ ). The relatively small number of patients with the SE probably resulted in the marginal significance. Nevertheless, for every 1% increase in European ancestry, the odds of having at least 1 SE allele increased by a factor of 1.020. Similar results were observed when the analysis was restricted to the 596 AIMs common to panels 1 and 2. Specifically, HLA–DRB1\*0401 was significantly related to European admixture (OR 1.037, 95% CI 1.003–1.071,  $P=0.0277$ ), and there was marginal association between the presence or absence of the SE and the percent European ancestry (OR 1.02, 95% CI 0.997–1.047,  $P=0.0895$ ).

### Association of autoantibody status with HLA–DRB1 alleles

Of the 286 CLEAR RA patients for whom baseline serum anti-CCP antibody and HLA–DRB1 genotypes were known, there was a significant association of the SE with the presence of the anti-CCP antibody. Among 176 patients with positive anti-CCP antibodies, 86 (48.9%) had at least 1 SE allele, compared with 36 (32.7%) of 110 patients for whom the anti-CCP antibody was negative ( $P=0.01$ , by chi-square test).

## DISCUSSION

This study provides strong evidence that HLA–DRB1 alleles associated with RA susceptibility in persons of European descent contribute to the disease in African Americans. The HLA–DRB1 SE is strongly associated with RA in African Americans (25.2% of patients with RA versus 13.6% of control subjects) but appears to have a smaller absolute effect than in persons of European ancestry, in which the proportion of patients with at least 1 SE allele is as high as 83% compared with 46% of controls (38). It remains unclear

whether these associations are attributable to the HLA-DRB1 alleles per se or to allelic variants in genetic regions in linkage disequilibrium with HLA-DRB1. There are a plethora of genes with immune function in the MHC locus, many of which have been implicated in RA, including HLA-DQA1, HLA-DQB1, tumor necrosis factor, MICB, and BTNL2 (39–41).

In our study, there was a relative paucity of \*1101 alleles in African American patients with RA compared with control subjects (allele frequency 5.5% in patients versus 13.3% in controls;  $P = 0.00004$ ). It has been hypothesized by other investigators that particular HLA-DRB1 alleles have a dominant protective effect, while the susceptibility to RA may be attributable to linked HLA-DQB1 alleles (42). The purported protective HLA-DRB1 alleles, \*0402, \*1301, and \*1302, encode the amino acid motif DERAA at residues 70–74 of the  $\beta$ -chain, while DRB1\*1101 encodes DRRAA. Rather than a protective effect of this allele, we speculate that this is more likely a reflection of a zero-sum effect, in which increases in the frequency of particular alleles (in this case those bearing the SE) must lead to decreases in the frequency of other alleles.

The data presented here are the first to show that genetic admixture of DNA from persons of European ancestry into Africans may contribute to the presence of a rheumatic disease-associated genotype. There appears to be a low prevalence of RA and a low allele frequency of HLA-DRB1\*04 alleles in West Africans (6). In contrast, RA is more common in African Americans, and our data show a higher frequency of HLA-DRB1\*04 alleles and other SE-containing alleles in African Americans than is reported in Africans. The proportion of SE-containing alleles, although higher in African Americans with RA than in controls, is lower than that reported in RA populations of European ancestry. Thus, these data are compatible with the hypothesis that HLA-DRB1 alleles containing the SE from European populations were admixed into the African American population, followed by an environmental (or other nongenetic) trigger, leading to RA (Figure 3).

In order to identify new susceptibility loci, we used AncestryMap software (37), using the same strategy that was reported in a recent study on admixture mapping of multiple sclerosis genes (33). We did not find evidence for association at any location in the genome, including the MHC locus on chromosome 6p21.3, most likely as a result of a very small sample size for an admixture mapping study. The analysis of 325 African Americans with RA in this study was estimated to have statistical power to detect loci where African or European ancestry on average confers a multiplicative increased risk of 2.0-fold (37). The absence of association of the MHC locus with RA in this sample suggests that the relative risk of RA attributable to HLA-DRB1 in African Americans is <2.0-fold. This finding also suggests that non-HLA genes may be the main admixture-dependent genetic determinants of RA risk in the African American population and are compatible with the smaller absolute effect of HLA-DRB1 alleles on RA susceptibility seen in African Americans compared with persons of European ancestry. In order to clarify this issue, we plan to carry out future admixture analyses of African Americans with RA, using the estimated total of 1,000 patients to be enrolled in the CLEAR registry.

This analysis confirms the finding in other ethnic groups that the SE appears to be associated with anti-CCP antibody-positive RA (32). There remain many unanswered questions with regard to the role of HLA-DRB1 alleles in RA among different ethnic groups. For example, these alleles are important in terms of the radiographic severity of RA in persons of European ancestry (10,43) and possibly in treatment response (44), but their influence on severity and treatment response in African Americans remains unknown. Similarly, the role of HLA-DRB1 alleles and genetic admixture in Asian, Native American, and other ethnic groups needs to be elucidated.



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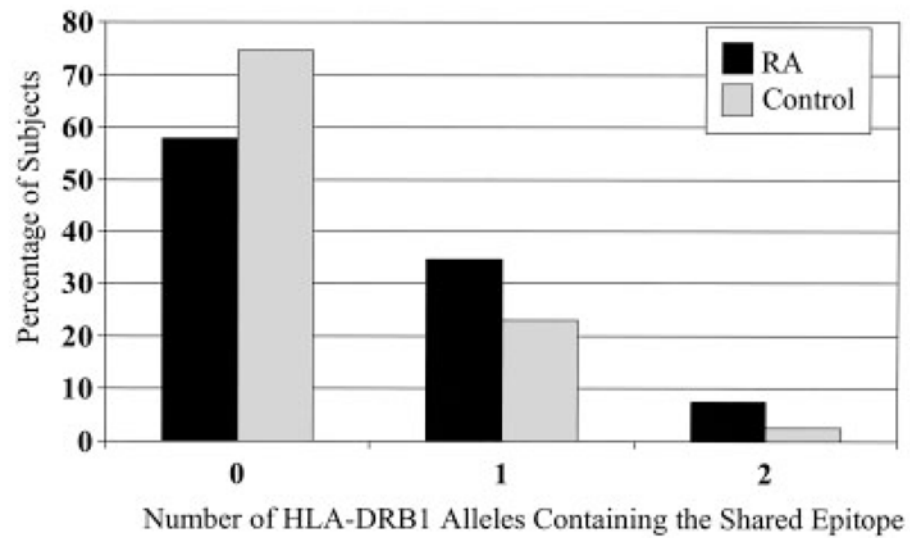
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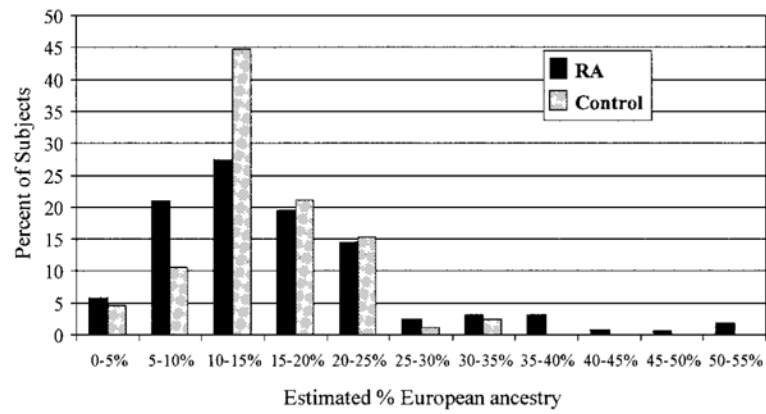
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## APPENDIX A: THE CLEAR INVESTIGATORS

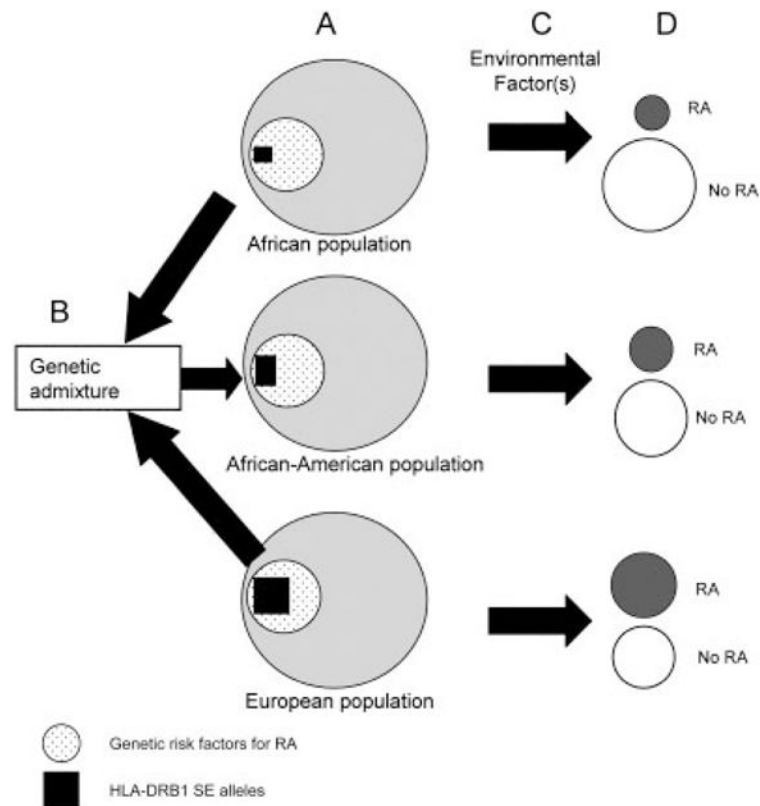
The CLEAR investigators are as follows: S. Louis Bridges, Jr., MD, PhD (Director), Larry W. Moreland, MD (Co-Director; current address: University of Pittsburgh), George Howard, DrPH (Co-Director), Graciela S. Alarcón, MD, MPH (University of Alabama at Birmingham); Doyt L. Conn, MD (Emory University); Beth L. Jonas, MD, Leigh F. Callahan, PhD (University of North Carolina); Edwin A. Smith, MD, Gary S. Gilkeson, MD (Medical University of South Carolina).



**Figure 1.** Percentage of African Americans with rheumatoid arthritis (RA) and African American control subjects, according to the number of HLA-DRB1 alleles containing the shared epitope ( $P=0.0007$ , RA versus control, by chi-square test).



**Figure 2.** Distribution of estimated percent European ancestry among African American patients with rheumatoid arthritis (RA) and control subjects in the Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis registry.



**Figure 3.** Theoretical model of the role of genetic admixture on risk of rheumatoid arthritis (RA) in African Americans. **A**, HLA-DRB1 shared epitope (SE) alleles are more common in the normal European population than in the normal African population. **B**, After being formed by genetic admixture of Europeans and Africans, the African American population has a higher proportion of SE-containing alleles than Africans, but a lower proportion than Europeans. **C** and **D**, When environmental or unknown factors trigger the disease, RA is more likely to develop in African Americans with SE alleles than in those without SE alleles.

**Table 1**

Baseline characteristics of 325 African American patients with RA in the CLEAR registry

Age at onset, mean $\pm$ SD years	51.2 $\pm$ 13.3
Disease duration, mean $\pm$ SD months	13.5 $\pm$ 7.2
Female sex	81
Rheumatoid factor positive	73
Anti-CCP antibody positive	62
Use of methotrexate at baseline visit	59.3
Use of other DMARD at baseline visit	22.0
Current cigarette smoking at baseline visit	33.9

\* Except where indicated otherwise, values are the percent. RA = rheumatoid arthritis; CLEAR = Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis; anti-CCP = anti-cyclic citrullinated peptide; DMARD = disease-modifying antirheumatic drug.

Table 2

Frequency of HLA-DRB1 alleles among African Americans with early RA and African American control subjects\*

Allele	No. (%) of RA patients (n = 321)	No. (%) of control subjects (n = 166)	No. (%) of healthy African Americans (n = 564)	P, RA versus control subjects (OR, 95% CI)	P, RA versus healthy African Americans (OR, 95% CI)
0101 <sup>‡</sup>	32 (5.0)	14 (4.2)	26 (2.3)		0.004 (2.22, 1.27–3.88)
0102 <sup>‡</sup>	26 (4.0)	18 (5.4)	49 (4.3)		
0103	1 (0.2)	2 (0.6)	2 (0.2)		
0301	36 (5.6)	27 (8.1)	79 (7.0)		
0302	33 (5.1)	22 (6.6)	83 (7.4)		
0303	1 (0.2)	0	1 (0.1)		
0401 <sup>‡</sup>	36 (5.6)	4 (1.2)	29 (2.6)	0.0004 <sup>‡</sup>	0.0018 (2.24, 1.33–3.81)
0402	1 (0.2)	0	1 (0.1)		
0403	2 (0.3)	1 (0.3)	2 (0.2)		
0404 <sup>‡</sup>	11 (1.7)	1 (0.3)	4 (0.4)	0.05 <sup>‡</sup>	0.006 (4.89, 1.44–18.26)
0405 <sup>‡</sup>	28 (4.4)	3 (0.9)	8 (0.7)	0.002 <sup>‡</sup>	<0.000001 (6.37, 2.76–15.27)
0407	0	1 (0.3)	6 (0.5)		
0408 <sup>‡</sup>	1 (0.2)	0	0		
0409	0	0	1 (0.1)		
0411	1 (0.2)	0	1 (0.1)		
0412	1 (0.2)	0	0		
0413 <sup>‡</sup>	0	0	0		
0414	0	1 (0.3)	0		
0701	54 (8.4)	18 (5.4)	102 (9.0)		
0801	1 (0.2)	0 (0)	9 (0.8)		
0802	4 (0.6)	0 (0)	2 (0.2)		
0804	30 (4.7)	17 (5.1)	62 (5.5)		
0806	4 (0.6)	2 (0.6)	3 (0.3)		
0811	1 (0.2)	1 (0.3)	2 (0.2)		
0901	25 (3.9)	7 (2.1)	31 (2.7)		
0903	1 (0.2)	0 (0)	0		
0905	1 (0.2)	0 (0)	0		



Allele	No. (%) of RA patients (n = 321)	No. (%) of control subjects (n = 166)	No. (%) of healthy African Americans (n = 564)	P, RA versus control subjects (OR, 95% CI)	P, RA versus healthy African Americans (OR, 95% CI)
1001 <sup>†</sup>	28 (4.4)	5 (1.5)	19 (1.7)	0.03 (2.98, 1.08–8.88)	0.0013 (2.66, 1.42–4.99)
1101	35 (5.5)	44 (13.3)	111 (9.8)	0.00004 (0.38, 0.23–0.62)	0.0016 (0.52, 0.35–0.79)
1102	17 (2.6)	10 (3.0)	45 (4.0)		
1103	1 (0.2)	0	0		
1104	5 (0.8)	0	5 (0.4)		
1110	3 (0.5)	0	0		
1114	1 (0.2)	0	0		
1201	28 (4.4)	15 (4.5)	36 (3.2)		
1202	2 (0.3)	2 (0.6)	4 (0.4)		
1301	29 (4.5)	18 (5.4)	67 (5.9)		
1302	25 (3.9)	18 (5.4)	76 (6.7)		
1303	25 (3.9)	8 (2.4)	39 (3.5)		
1304	10 (1.6)	8 (2.4)	14 (1.2)		
1305	0	0	1 (0.1)		0.02 (0.55, 0.34–0.91)
1311	0	0	1 (0.1)		
1312	1 (0.2)	0 (0)	0		
1331	0	0	1 (0.1)		
1401	7 (1.1)	8 (2.4)	15 (1.3)		
1402 <sup>†</sup>	0	0	3 (0.3)		
1404	0	1 (0.3)	2 (0.2)		
1421	0	1 (0.3)	0		
1501	12 (1.9)	7 (2.1)	28 (2.5)		
1502	4 (0.6)	2 (0.6)	2 (0.2)		
1503	66 (10.3)	39 (11.7)	138 (12.2)		
1601	1 (0.2)	0	0		
1602	11 (1.7)	7 (2.1)	18 (1.6)		
Total <sup>§</sup>	642 (100)	332 (100)	1,128 (100)		

\* Data on the rheumatoid arthritis (RA) patients are from the Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis (CLEAR) registry, and data for the control subjects are from the CLEAR registry and from work site volunteers combined. Two control subjects had 1 ambiguous HLA-DRB1 allele and were excluded from the analysis. Data on healthy work site volunteers are from ref. 25. OR = odds ratio; 95% CI = 95% confidence interval. Only *P*-values that were significant are shown.

<sup>†</sup> Alleles containing the shared epitope.

<sup>‡</sup> Calculated by Fisher's exact test; all other values were calculated using the chi-square test.

<sup>§</sup> Values are the no. (%) of alleles.

Table 3

Distribution of subjects based on the number of HLA-DRB1 alleles with the SE and the frequency of HLA-DRB1 alleles with and without the SE\*

	No. of SE alleles					P, RA vs. controls	SE alleles	Non-SE alleles	P, RA vs. controls (OR, 95% CI)
	0	1	2	2	2				
CLEAR RA patients (n = 321)	186 (57.9)	111 (34.6)	24 (7.5)	–	–	162 (25.2)	480 (74.8)	–	
Combined controls (n = 166)	124 (74.7)	38 (22.9)	4 (2.4)	0.0007	–	45 (13.6)	287 (86.4)	0.003 (2.15, 1.48–3.14)	
CLEAR controls (n = 116)	82 (70.7)	30 (25.9)	4 (3.4)	0.04	–	37 (15.9)	195 (84.1)	0.005 (1.78, 1.18–2.66)	
Work site volunteers (n = 50)	42 (84.0)	8 (16.0)	0 (0)	0.001	–	8 (8.0)	92 (92.0)	0.0002 (3.88, 1.78–1.98)	
Healthy African Americans (n = 564)	–	–	–	–	–	138 (12.2)	988 (87.6)	<0.000001 (2.41, 1.86–3.10)	
Combined CLEAR, work site, and healthy African American controls (n = 730)	–	–	–	–	–	183 (12.5)	1,275 (87.3)	<0.000001 (2.35, 1.84–3.00)	

\* Values are the number (%). Data on healthy African Americans are from ref. 25. All calculations were done by chi-square analysis; SE = shared epitope; RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval; CLEAR = Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis.

**Table 4**

Distribution of European ancestry stratified by HLA-DRB1 SE status and number of subjects with 0, 1, or 2 SE alleles\*

	No. of SE alleles			SE present
	0	1	2	
RA patients (panel 1)				
Mean ± SD	15.1 ± 8.9	16.2 ± 9.0	19.2 ± 11.9	16.7 ± 9.6
No.	181	109	23	132
Controls (panel 2)				
Mean ± SD	14.3 ± 5.7	16.4 ± 4.4	15.6 ± 3.2	16.3 ± 4.2
No.	61	18	3	21
Combined RA patients and controls <sup>†</sup>				
Mean ± SD	14.9 ± 8.2	16.2 ± 8.5	18.8 ± 11.3	16.7 ± 9.1
No.	242	127	26	153
Combined RA patients and controls <sup>‡</sup>				
Mean ± SD	14.7 ± 8.3	16.4 ± 8.3	18.2 ± 11.4	16.7 ± 8.9
No.	242	127	26	153

\* Values are the mean ± SD percent European admixture along with the no. of subjects. SE = shared epitope; RA = rheumatoid arthritis.

<sup>†</sup> Results obtained using all data from panel 1 for RA patients and all data from panel 2 for controls.

<sup>‡</sup> Results obtained using only the 596 ancestry-informative markers common to panels 1 and 2.

Table 5

Association of percent European admixture with HLA-DRB1 SE status, using additive genetic effects *t*-tests and Spearman's rank order rho tests\*

	Additive genetic effects			Spearman's rank order		
	df	<i>t</i>	<i>P</i>	$\rho$	<i>P</i>	<i>P</i>
RA patients (panel 1)						
No. of SE alleles (0, 1, or 2)	311	1.96	0.05	0.1017	0.07	
SE absent versus present	311	1.53	0.13	0.0907	0.11	
Controls (panel 2)						
No. of SE alleles (0, 1, or 2)	80	1.30	0.20	0.2267	0.04	
SE absent versus present	80	1.47	0.15	0.2308	0.04	
Combined RA patients and controls <sup>†</sup>						
No. of SE alleles (0, 1, or 2)	393	2.37	0.02	0.1189	0.02	
SE absent versus present	393	1.98	0.05	0.1112	0.03	
Combined RA patients and controls <sup>‡</sup>						
No. of SE alleles (0, 1, or 2)	393	2.52	0.01	0.1430	0.004	
SE absent versus present	393	2.33	0.02	0.1405	0.005	

\* SE = shared epitope; RA = rheumatoid arthritis.

<sup>†</sup> Results obtained using all data from ancestry-informative marker (AIM) panel 1 for RA patients and all data from AIM panel 2 for controls.

<sup>‡</sup> Results obtained using only the 596 ancestry-informative markers common to AIM panels 1 and 2.

**Table 6**

Association of percent European admixture with HLA-DRB1 SE status but not disease status \*

	df	t	P
AIM panels 1 and 2 <sup>†</sup>			
No. of SE alleles (0, 1, or 2)	392	2.44	0.02
RA versus control	392	0.60	0.55
AIM panels 1 and 2 <sup>†</sup>			
SE absent versus present	392	1.88	0.06
RA versus control	392	0.64	0.52
596 common AIMs <sup>‡</sup>			
No. of SE alleles (0, 1, or 2)	392	2.38	0.02
RA versus control	392	0.84	0.40
596 common AIMs <sup>‡</sup>			
SE absent versus present	392	2.19	0.03
RA versus control	392	0.85	0.39

\* SE = shared epitope; RA = rheumatoid arthritis.

<sup>†</sup> Results obtained using all data from ancestry-informative marker (AIM) panel 1 for RA patients and all data from AIM panel 2 for controls.

<sup>‡</sup> Results obtained using only the 596 AIMs common to AIM panels 1 and 2.