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## Association of Single Nucleotide Polymorphisms (SNPs) in *CCR6*, *TAGAP* and *TNFAIP3* with Rheumatoid Arthritis in African Americans

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### Abstract

**Objective**—We previously reported an analysis of single nucleotide polymorphisms (SNPs) in three validated European rheumatoid arthritis (RA) susceptibility loci, *TAGAP*, *TNFAIP3*, and *CCR6* in African-Americans with RA. Unexpectedly, the disease-associated alleles were different in African-Americans than in Europeans. In an effort to better define their contribution, we performed additional SNP genotyping in these genes.

**Methods**—Seven SNPs were genotyped in 446 African Americans with RA and 733 African American controls. Differences in minor allele frequency between cases and controls were analyzed after controlling for global proportion of European admixture, and pairwise linkage disequilibrium (LD) was estimated among the SNPs.

**Results**—Three SNPs were significantly associated with RA: *TNFAIP3* rs719149 A allele (OR (95% CI) 1.22 (1.03–1.44) ( $p = 0.02$ ); *TAGAP* rs1738074 G allele OR 0.75 (0.63–0.89), ( $p = 0.0012$ ); and *TAGAP* rs4709267 G allele 0.74 (0.60–0.91), ( $p = 0.004$ ). Pairwise LD between the *TAGAP* SNPs was low ( $R^2 = 0.034$ ). The haplotype containing minor alleles for both *TAGAP* SNPs was uncommon (4.5%). After conditional analysis on each *TAGAP* SNP, its counterpart remained significantly associated with RA (rs1738074 for rs4709267  $p = 0.00001$ ; rs4709267 for rs1738074  $p = 0.00005$ ), suggesting independent effects.

**Conclusions**—SNPs in regulatory regions of *TAGAP* and an intronic SNP (*TNFAIP3*) are potential susceptibility loci in African Americans. Pairwise LD, haplotype analysis, and SNP conditioning analysis suggest that these two SNPs in *TAGAP* are independent susceptibility

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We have no conflicting interests involved in this project.

alleles. Additional fine mapping of this gene and functional genomic studies of these SNPs should provide additional insight into the role of these genes in RA.

Rheumatoid arthritis (RA) is an autoimmune disease that affects 0.5–1% of the population worldwide and is characterized by inflammation of the synovial joints. This disease can be subdivided phenotypically based on the presence or absence of autoantibodies such as rheumatoid factor (RF) or autoantibodies to cyclic citrullinated peptide (anti-CCP). It is known that genetic and environmental factors play a role in the disease, but the cause remains unknown. Several studies conducted in persons of European descent have shown at least 20 common risk alleles for RA (1, 2); however, few studies have been done in persons of African ancestry (3). A previous study from our group (3) found that the odds ratio (OR) of association of the risk alleles seen in persons of European descent were similar in African Americans with three exceptions: T-cell activation RhoGTPase activating protein (*TAGAP*), tumor necrosis factor, alpha-induced protein 3 (*TNFAIP3*), and chemokine (C-C motif) receptor 6 (*CCR6*), which all had OR in opposite directions when compared to Europeans (alleles associated with RA in patients of European ancestry were less common in African-American RA patients than in African-American controls). We believe that this could be caused by different LD or allele frequencies between the populations.

*TAGAP* is known to be involved in T-cell activation and has been implicated in several autoimmune diseases, including celiac disease and RA (4). *TNFAIP3* has been found to limit inflammation and perform as an apoptosis mediator (5). This gene has been implicated in various autoimmune diseases including RA. *CCR6* is believed to be involved in leukocyte recruitment and inflammatory response. It has been implicated in cancer, Graves' disease, psoriasis, and RA (6). To further characterize genetic variation in these genes for RA susceptibility, we report results from SNP genotyping in the exons and regulatory regions of *TAGAP*, *CCR6* and *TNFAIP3* (Table 1).

## PATIENTS AND METHODS

### Study Subjects

We analyzed seven SNPs in 466 autoantibody (either RF or anti-CCP antibody positive) African American RA patients and 733 healthy African American controls. All patients with RA were participants in the Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis (CLEAR) Registry, with samples collected as previously described (3). Healthy African American controls were similar to patients in sex, age, and geographic location. Of the 733 controls, 303 were from the CLEAR Registry; the remaining controls were recruited from the Birmingham, Alabama area.

### Genotyping

We selected SNPs in regulatory regions, exons, and introns. Genotyping was performed using an Applied Biosystems 7900HT sequencing system and Applied Biosystems Taqman SNP genotyping assays. For quality control purposes, we required that each SNP meet the following criteria: a missing-genotype rate of <10%, a minor allele frequency (MAF) of >%, and Hardy-Weinberg equilibrium (defined by  $P > 0.001$ ).

### Ancestry Informative Markers (AIMs)

To control for the potentially confounding effect of population structure, individual admixture estimates were made among the study participants from genotypes at ancestry informative markers (AIMs). DNA samples from CLEAR RA cases and controls were genotyped using a custom Illumina chip with 3,317 AIMs in the laboratory of Dr. Peter Gregersen as part of the International MHC and Autoimmunity Genetics Network

(IMAGEN). The percent global European ancestry for each participant was calculated based on the AIM genotypes using the software package Structure Version 2.3.1(7). Simulations were run assuming two founding populations, 10,000 burn-ins, and 1,000 subsequent replicates to generate the estimates.

### Statistical Analysis

Allele frequency differences between cases and controls were evaluated using 2×2 contingency table analysis and tests of association were performed using Pearson's chi square. In order to address potential confounding due to population structure, we also performed a logistic regression analysis in which the response was case or control and the explanatory variables were proportion global European admixture and SNP genotype (0, 1, or 2 copies of the minor allele). To determine if the effects of significantly associated SNPs correlated with each other, logistic regression models were fit to both markers of interest while testing the significance of each and conditioning on the other. Pairwise LD between SNPs is reported as  $r^2$ . All analyses were run using PLINK v1.06 (8). Haplotype frequencies were inferred using PHASE (9).

## RESULTS

The allele frequencies for each SNP tested for association with risk for RA are shown in Table 1. Of the seven SNPs analyzed, three alleles were statistically different between RA patients and controls: *TNFAIP3* rs719149 A allele (OR and 95% CI) 1.22 (1.03–1.44) ( $p=0.02$ ); *TAGAP* rs1738074 G allele, OR 0.75 (0.63–0.89), ( $p=0.0012$ ); and *TAGAP* rs4709267 G allele OR 0.74 (0.60–0.91), ( $p=0.004$ ). As expected, pairwise LD between SNPs from different genes (*TNFAIP3* and *TAGAP*) was very low. The maximum  $R^2$  was 0.362 between rs3093008 and rs10946217 in *CCR6*, indicating modest levels of LD among these SNPs. It is notable that the two SNPs in *TAGAP* had an  $R^2$  of 0.034, indicating weak linkage disequilibrium between them. Haplotype analyses of these two SNPs revealed the haplotype (GG) containing both minor alleles was the least frequently observed haplotype (4.5%).

We performed logistic regression to control for the effect of *TAGAP* rs4709267 and found that the association of *TAGAP* rs1738074 remained statistically significant ( $p=0.00001$ ). Likewise, when we controlled for the effect of rs1738074, the association of rs4709267 remained statistically significant ( $p=0.00005$ ). The minor allele frequencies for all SNPs were consistent with those reported for African Americans or for the weighted average for Sub-Saharan African (80%) and European (20%) allele frequency reported in dbSNP(10) (Table 1).

## DISCUSSION

While there have been many genome-wide association (GWA) studies performed in persons of European or East Asian descent with RA, few studies have genotyped putative risk alleles in African Americans. Our previous work showed that SNPs in *TAGAP*, *CCR6*, and *TNFAIP3* are associated with risk for RA in African Americans. In the current study, we found that *TNFAIP3* rs719149 appeared to be associated with risk for RA, and that *TAGAP* rs1738074 and rs4709267 alleles associated with RA in European ancestry populations appear more frequently in African-American controls than RA patients. Of the 7 SNPs analyzed in this study, only two have been previously reported in other RA studies: *TNFAIP3* rs2230923 and *TAGAP* rs1738074. *TNFAIP3* rs2230923 was found to be associated with RA in persons of European ancestry (OR and 95% CI: 1.82 (1.05–3.18),  $p=0.025$ )(11) and in persons of Japanese ancestry (OR and 95% CI: 1.35 (1.18–1.56),  $p=0.000026$ )(12).

Given the effect of *TNFAIP3* rs2230923 in Europeans (OR 1.85), our study had sufficient power to detect an effect in African-Americans. However, we saw no significant association of RA with this SNP, perhaps due to differences in minor allelic frequency between African (YRI) (0.45) and European (CEU) populations (0.03). The *TAGAP* rs1738074 SNP was previously reported to be associated with RA in persons of European ancestry: OR(95% CI): 0.90 (0.86–0.95),  $p=0.00017$ (13). The effect of this SNP appeared to be similar in our population (OR=0.75,  $p=0.0012$ ). Of note, the minor allele in the European population is A, but in African-Americans is G. Thus, the minor allele is associated with RA in each population, but in one case it is the A allele (European) and in the other it is the G allele (African-American). This SNP may be a component of a novel risk haplotype for RA that can be seen across multiple ethnicities. The *TNFAIP3* rs719479 SNP appears to confer risk in our population, but information is needed on this SNP in other populations with RA to determine if could be part of a risk haplotype.

Based on the low LD score ( $R^2=0.034$ ) in combination with the low haplotype frequency of the minor alleles (4.5%) and the results of the logistic regression, *TAGAP* SNPs rs4709267 and rs1738074 appear to be independent risk alleles, which suggests that multiple haplotypes spanning *TAGAP* could be involved. Fine mapping studies have been performed in *TAGAP* (13) and *TNFAIP3* (14) in European ancestry populations; similar studies in African Americans may yield additional insights. Future studies in European and Asian populations, and in an independent cohort of African Americans would be helpful for comparing risk allele frequencies and LD structure between the populations.

Given that *TAGAP* is involved in T-cell activation and *TNFAIP3* is involved in limiting inflammation, changes in these genes may be very important in autoimmune diseases. RA-associated SNPs in *TAGAP* may be involved in altering the threshold of T-lymphocyte activation, thus affecting the development of autoimmunity. The SNP in *TNFAIP3* appears to confer risk, so perhaps this SNP limits the ability of the protein to abrogate the inflammatory response. The importance of these SNPs should be further investigated by functional analysis to examine their biologic effects and thus their potential role in the pathogenesis of RA.

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Table 1

The allele frequencies, odds ratio (OR), 95% confidence interval (CI), and p-values for the seven SNPs characterized in this study, controlled for population structure.

Gene, SNP	Region	MAF-CEU/YRI/AFR-dbsNP(10)	MAF-African-American RA	MAF-African-American control	OR (95% CI)	p-value
<b>CCK6</b>						
rs3093008 A/G*	5 UTR	0.004/0.42/0.25	0.30	0.29	1.01(0.84-1.21)	0.9325
rs3093007 C/T	Exon (syn)	0.16/0.16/0.22	0.17	0.18	0.96(0.77-1.20)	0.708
rs10946217 A/T	3 UTR	0/0.35/0.23	0.29	0.26	1.14(0.94-1.36)	0.18
<b>TAGAP</b>						
rs4709267 A/G	3 UTR	0.10/0.20/0.20	0.17	0.23	0.74 (0.60-0.91)	0.004
rs1738074 A/G	5 UTR	0.54/0.31/0.29	0.29	0.36	0.75(0.63-0.89)	0.0012
<b>TNFAIP3</b>						
rs719149 A/G	Intron	0.90/0.40/0.48	0.48	0.43	1.22 (1.03-1.44)	0.02
rs2230926 G/T	Exon (missense)	0.03/0.45/0.37	0.37	0.38	0.97(0.82-1.15)	0.759

MAF=minor allele frequency. CEU=Caucasian. YRI=Sub-Saharan African. AFR=African American.

\* minor allele frequency for African ancestry is shown in bold.