

# HLA Class II and TNF Genes in African Americans From the Southeastern United States: Regional Differences in Allele Frequencies

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**ABSTRACT:** Knowledge of population major histocompatibility complex gene frequencies is important for construction of organ donor pools and for studies of disease association. Human leukocyte antigen DRB1 (HLA-DRB1), HLA-DQB1, and TNF $\alpha$ -308 (G-A) promoter genetic typing was performed in 112 healthy, unrelated African Americans (AAs) from the southeastern United States. Allele frequencies were compared with published frequency data from other AA populations. Our AA population had the highest frequency of HLA-DRB1\*09 (6.7%) reported in any AA population. The frequency of the TNF $\alpha$ -308A polymorphism was also high (14.4%), when compared with published frequencies in AAs. Significant regional differences in the distribution of most HLA-DRB1 and HLA-DQB1 alleles were observed in all AA populations examined. The AA HLA-DRB1 and

-DQB1 frequencies also differed from published Caucasian frequencies. This is the first report describing the distribution of TNF $\alpha$  promoter alleles in the Southeastern United States. The high DRB1\*09 and TNF $\alpha$ -308A allele frequencies of our population most resemble the frequencies of these alleles in certain West African populations. These varying major histocompatibility complex gene frequencies may reflect different regional population structures among AAs in the United States, which may be due to differences in ancestral origins, migration, and racial admixture. *Human Immunology* 64, 639–647 (2003). © American Society for Histocompatibility and Immunogenetics, 2003. Published by Elsevier Inc.

**KEYWORDS:** genetics; polymorphism; cytokine; allele; DNA typing

## ABBREVIATIONS

AA African American  
ASHI American Society for Histocompatibility and Immunogenetics  
DNA deoxyribonucleic acid  
EBV Epstein-Barr virus  
HCW histocompatibility workshop  
HLA human leukocyte antigen  
HWE Hardy Weinberg equilibrium  
IDDM insulin-dependent diabetes mellitus

NS not significant  
NYC New York City  
PBMC peripheral blood mononuclear cells  
RA rheumatoid arthritis  
SE shared epitope  
SLE systemic lupus erythematosus  
SSP sequence-specific primers  
TNF $\alpha$  tumor necrosis factor alpha  
USA United States of America

## INTRODUCTION

Many studies have found certain human leukocyte antigens (HLA) to be associated with some autoimmune diseases [1], graft survival after transplantation [2], sus-

ceptibility to some infectious diseases [3, 4], and to influence vaccine responsiveness [5–9]. Racial differences in these associations, which may reflect differences in

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population frequencies of HLA antigens, have also been reported. For example different HLA Class II alleles appear to be strongly associated with rheumatoid arthritis (RA) among different racial/ethnic groups. These include DRB1\*0401 and DRB1\*0404 in Caucasians [10, 11], DRB1\*0405 in Japanese [12, 13], DR1 and DR10 in Israelis [14], and DR9 in Chileans [15]. As another example, HLA-DR2 is associated with susceptibility to tuberculosis in many populations [16, 17], but in Cambodians this susceptibility association is with DQB1\*0503 and not with HLA-DR2 [18]. HLA class II genes are highly polymorphic, with the number of alleles ranging from 2 at the DRA, to 44 at the DQB1, and to > 200 at the DRB1 locus. These multiple alleles cannot all be distinguished by immunologic methods. Accurately defining population HLA frequencies and identifying HLA disease associations must rely on molecular typing. To date, most studies of HLA Class II allele frequencies that have used molecular resolution techniques have been conducted among European, Asian, and Caucasian American populations. Few such studies have included African Americans (AAs).

Because of their diverse ancestral origins, admixture with other ethnic groups, migration patterns, and the effects of selective pressures subsequently encountered throughout approximately 20 generations in the western hemisphere, AAs would be expected to possess considerable genetic diversity. A study by Acton *et al.* [19], using serologic typing and mixed lymphocyte reaction (MLR), reported significant differences in the distribution of HLA-DR phenotypes among AA participants in Alabama compared with those from Maryland, with significantly reduced frequencies of DR2, DR6, and DR7 but a significantly increased frequency of DR10 in the Alabama population. Just *et al.* [20], using molecular typing methods to type nine loci in the HLA class II region among 241 AAs from New York City (NYC), observed a higher allelic diversity at the DRB1 and DPB1 loci in this population compared to Europeans, suggesting that admixture may account for some of this diversity. This increased genetic diversity decreases the opportunity for finding well matched, unrelated organ donors for transplant patients and could explain why graft survival among AAs who receive AA donor organs has been reported to be lower than that among Caucasian Americans [21]. Further studies of HLA allele frequencies in AAs, particularly in different regions of the United States, may provide valuable information not only about ancestral origins, migration patterns, and degree of admixture but also for the construction of organ donor pools for different geographical locations.

In this study we used molecular techniques to determine HLA-DRB1 and DQB1 frequencies in healthy

AAs. The patients were all residents of the southeastern United States, and were HLA typed as controls for a multigenic study of RA in AAs or for organ transplant donation. We also determined the frequencies of the TNF $\alpha$  -308 (G/A) promoter polymorphism because this cytokine has been implicated in the pathogenesis of RA, insulin-dependent diabetes mellitus (IDDM) and systemic lupus erythematosus (SLE) [22–24]. Moreover, several groups, using reporter gene assays, have found that the TNF $\alpha$  -308A promoter is a stronger transcriptional activator compared to the TNF $\alpha$  -308G allele [25, 26].

## MATERIALS AND METHODS

### Patients

One hundred twenty unrelated healthy AA blood donors were enrolled; 59 were from the healthy blood donor pool at the Emory University Hospital HLA typing laboratory. These participants were relatives of transplant recipients, self-reported to be AA and residents of southeastern states. All were healthy and none had IDDM or other autoimmune diseases. Data on racial admixture was not available for these 59 donors. Of the remaining 61 participants, 45 were recruited through the blood donor program of the Scientific Resources Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA), and 16 were recruited through Grady Memorial Hospital and Emory University Hospital. These latter 61 persons, who were all Georgia residents, were interviewed and asked to complete a questionnaire that established absence of any autoimmune disease. Participants recorded a three generation race and ethnicity history for themselves, their parents, and their grandparents, and their own country of birth. Racial/ethnic choices on the questionnaire were: White, Black, Native American/Alaska Native, Asian/Pacific Islander, other, do not know, or no answer/refused. For this study AAs were defined as participants who recorded their race as Black and were born in the USA or its territories. Of the 61 controls interviewed, 45 provided race/heritage data. Of these 45, 38 (84%) reported only AA race/ethnicity for themselves and their preceding two generations. The other 7 (16%) reported racial/ethnic admixture with Native American or White in their parents and/or grandparents. Of the 120 enrolled, DNA availability resulted in 112 participants being HLA typed and 59 being TNF typed. All consent documents, protocols, questionnaires and laboratory procedures were approved by the institutional review boards of Emory University and CDC.

### Isolation of Peripheral Blood Mononuclear Cells, Epstein-Barr Virus Transformation, and Preparation of DNA

Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Hypaque density gradient centrifugation and were cryopreserved or used to generate Epstein-Barr virus (EBV) transformed B-cell lines (at the Emory University Hospital Clinical Research Center) using a standard procedure [27]. Genomic DNA was extracted from peripheral blood mononuclear cells or B-cell lines using either the Genomix Cell Kit (Washington Biotechnology, Inc., Bethesda, MD, USA) or the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA), according to the manufacturer's instructions.

### HLA-DRB1 and HLA-DQB1 Typing

The HLA-DRB1, -DRB3, -DRB4, -DRB5, and -DQB1 alleles were determined in 112 donors by using polymerase chain reaction amplification with sequence-specific primers (PCR-SSP; UCLA Mixtures, UCLA Tissue Typing Laboratory, Los Angeles, CA, USA) [28]. Typing was performed according to the manufacturer's instructions, with the following modifications: cycling parameters were changed to 32 cycles each consisting of 15 seconds denaturation at 94 °C, annealing at 58 °C for 15 seconds, and extension at 73 °C for 10 seconds. The  $\beta$ -actin gene was amplified in each reaction as an internal DNA control, using the primers 5' AAGAGAGGCATCCTCACCT 3' and 5' CATCTCTTGCTCGAAGTCCA 3'. Genomic DNA was amplified in 24 amplification mixtures, and PCR products were subsequently separated on a 3% low-melting temperature agarose (NuSieve; FMC BioProducts, Rockland, ME, USA) and stained with ethidium bromide. HLA allele sets were identified by the band sizes and patterns observed. Resolution for all DRB1 alleles ranged from high to low depending on the number of new alleles reported in the literature during the duration of the study. Because of this variability and for the purpose of this analysis, alleles were grouped according to the first two digits of the DRB1\* designation. HLA-DRB1 alleles tested were as follows, with unresolved alleles in parenthesis: DRB1\*0101-04 (0102/04), \*0301-08, \*0401-27 (\*0401/08/16, 0404/13, 0405/09, 0415/22/25 and 0418/21), \*0701, \*0801-11 (\*0801/05, 0802/11, 0807/08/09), 09012, 1001, 1101-13 (\*1103/07, 1104/06, 1110/12), \*1201-04 (\*1201/03), \*1301-13 (\*1301/06/10, \*1309/11, \*1303/12/13), \*1401-17 (\*1401/05/08, \*1402/09/13, \*1404/11), \*1501-04 (\*1501/03/04), and 1601-06 (\*1601/03/04, \*1602/05/06). Because the typing method could not resolve DRB1\*0401/08/16, DRB1\*0405/09, DRB1\*0404/13, and DRB1\*0102/04, bands potentially identifying one of these alleles were classified as the first allele of the group. However, among AA RA patients

from the same population as these controls whose alleles were typed to high resolution using reverse dot blot and PCR-SSP, we did not identify DRB1\*0416/08/09/13 or DRB1\*0104 (McNicholl *et al.*, manuscript in preparation), suggesting that these alleles are rare in this population and thus the first allele assignments indicated above are likely to be correct. The RA-associated shared epitope (SE) was defined by the presence of HLA-DRB1\*0101, \*0102, \*0401, \*0404, \*0405, \*1402, and \*1001.

Medium- to-high resolution typing for DQB1 was performed using PCR-SSP (UCLA Mixtures, UCLA Tissue Typing Laboratory) [28]. For analysis, alleles were also grouped according to the first two digits of the DQB\* designation. DQB1 alleles tested were as follows (unresolved alleles in parenthesis): DQB1\*0201/02, 0301-05 (\*0302/03), 0401/02, 0501-04, 0601-09 (\*0605/09, \*0602/03). All lots of UCLA primers were tested for quality assurance at the Emory University Hospital Transplantation Laboratory against a panel of donor DNA of known DRB and DQB specificity.

### TNF $\alpha$ Promoter Typing

A 107 base pair (bp) region of the TNF $\alpha$  promoter was PCR amplified using the sense primer 5'AGGCAAT-AGGTTTTGAGGGCCAT 3' and the antisense primer 5' TCCTCCCTGCTCCGATCCCG 3' as described by Wilson *et al.* [29]. The G (TNF1 allele) to A (TNF2 allele) transition polymorphism at position -308 of the TNF $\alpha$  promoter was screened by digestion with NcoI enzyme (New England BioLabs, Boston, MA, USA), and the fragments were analyzed on an ethidium-bromide stained 3% low-melting temperature agarose gel (NuSieve, FMC BioProducts). Positive and negative controls were included with each batch of samples. Treatment of the TNF $\alpha$  -308G wild-type allele with NcoI gave two fragments of 87 bp and 20 bp, whereas the TNF $\alpha$  -308A allele was undigested and remained a single 107 bp fragment. The TNF $\alpha$  -308 promoter polymorphism was only determined in 59 subjects as DNA was not available from the other donors.

### Database Management and Statistical Analyses

Data (16 provided no answer) were managed and stored using the EpiInfo program (V6.04; CDC, Atlanta, GA). Allele frequencies for DRB1, DQB1, and TNF $\alpha$  were determined using twice the number of donors as the denominator. Hardy-Weinberg equilibrium (HWE) testing, linkage disequilibrium testing, and estimates of haplotype frequencies were performed using Arlequin software [30]. Because neither family studies nor sequencing was performed, haplotypes identified in this study are likely haplotypes based on mathematical likelihoods. Bernstein's formula [31] 'geno'=1-SQRT(1-

'pheno'), where SQRT represents the square root function, was used to calculate genotype frequencies from publications reporting only phenotype frequencies.

## RESULTS

### HLA Class II DRB1 and DQB1 Genes and DRB1-DQB1 Haplotypes in Southeastern AAs

The HLA-DRB1 genotypes were in HWE ( $p > 0.05$ ). African-Americans in our study had high frequencies of DRB1\*13 (20.5%), with the next most prevalent groups being DRB1\*15 (13.8%), DRB1\*03 (12.5%), and DRB1\*11 (12.0%), illustrated in Table 1 (first column). The frequencies of the DR3 antigen splits, DR17 and DR18, were equal in our population (data not shown). The frequency of DRB1\*09 was 6.7%. The frequency of the DRB1\*04 group, was 4.9%. Among the DR4 alleles, DRB1\*0405 was the most frequent (36.4%), followed by DRB1\*0404, DRB1\*0401, and DRB1\*0407 (18% each). When alleles were classified into the group comprising the RA-associated SE, 9.8% of AA carried an SE-encoding allele. The most frequent heterozygote DRB1 combination was DRB1\*15/DRB1\*13 (5.4%).

Alleles at the DQB1 locus were in HWE. The most frequent DQB1 group was 06 (29.5%), followed by DQB1\*02, \*03 and \*05. DQB1\*04 was least frequent (Table 1). DQB1\*0301 was the most frequent likely DQB1\*03 subtype. The most frequent likely DRB1-DQB1 haplotypes were DRB1\*1503 (01,04)/DQB1\*0602 (03), DRB1\*0701/DQB1\*0201 (02), and DRB1\*1201(03)/DQB1\*0501, with frequencies of 7.9%, 7.5%, and 5.0%, respectively. These haplotypic frequencies may be overestimates as not all alleles were resolved.

### TNF $\alpha$ Promoter Genotypes and DRB1-TNF and DQB1-TNF Haplotypes Among AAs

The TNF $\alpha$  promoter polymorphism was typed in a subset of 59 donors, who had available DNA; all were from Georgia. The distribution of HLA-DRB1 allele groups in this subset was not significantly different when compared with the total population of 112 donors. In particular, the frequency of DRB1\*0301 was similar in both groups (8.82% vs 6.25%, respectively,  $p = 0.54$ ). The frequency of the TNF $\alpha$  -308A allele was 14.4% (Table 2). Twenty-five percent of these controls were heterozygous, and 1.7% were homozygous for the G to A polymorphism. Both TNF alleles were in HWE. Because TNF $\alpha$  -308 and DRB1\*0301 are in linkage disequilibrium in some populations [32, 33], we examined this relationship in our population and found no significant linkage between the two ( $p = 0.13$ ). The most frequent likely DRB1-TNF $\alpha$  haplotypes were DRB1\*0701 (02-06)/TNF $\alpha$ -308G, DRB1\*1501 (03,04)/TNF $\alpha$ -308G,

DRB1\*0901(02)/TNF $\alpha$  -308G, and DRB1\*1501 (03,04)/TNF $\alpha$  -308A, with frequencies of 3.73%, 3.14%, 2.7%, and 1.4%, respectively. The most frequent likely DQB1-TNF $\alpha$  haplotypes were DQB1\*0301/TNF $\alpha$  -308G, DQB1\*0201 (02)/TNF $\alpha$  -308G, DQB1\*0501/TNF $\alpha$  -308G, and DQB1\*0201 (02)/TNF $\alpha$  -308A, with frequencies of 7.9%, 7.5%, 4.9%, and 2.9%, respectively. These haplotype frequencies could be overestimates, depending on the frequencies of the nonresolved HLA alleles.

## DISCUSSION

This is the first report of HLA class II and TNF-308 frequencies in healthy AAs from Georgia and other southeastern states. We observed significant differences in the distribution of DR and DQ antigen groups and alleles in this population compared to published frequencies in other AA populations. This was most notable for DRB1\*09. Our study also suggests that there may be regional differences in TNF $\alpha$  promoter allele frequencies in AAs.

### Comparisons With Seven Other AA Populations at the HLA-DRB1 Locus

The populations chosen for comparison had been HLA typed using molecular methods and included the following: 241 AAs from NYC [20]; 132 North American Blacks (reported at the 11<sup>th</sup> International Histocompatibility Workshop [IHW]) [34]; 86 AAs residing in Illinois (mainly in Chicago and surrounding suburbs); 64 cadaveric AA donors typed at the Transplantation Society of Michigan (Ann Arbor, MI), 69 AA random US blood donors (Dallas study) [35]; and 541 unrelated AAs from multiple US regions (reported at an ASHI Minority Antigens Workshop) [36]. Also included were 454 AAs from Alabama who were HLA-DR phenotyped using a microdroplet lymphocytotoxicity assay [19] because this is the only other study of an AA population from a geographically well-defined southeastern US region. For multiple analyses, Bonferroni corrections were not performed. Therefore, the statistical differences noted may not be significant and should be confirmed in other studies.

The DRB1\*01 allele frequency of 4.5% in our population was significantly higher than in the Dallas ( $p = 0.03$ ) and Alabama ( $p = 0.04$ ) populations but similar to frequencies reported in the other studies (Table 1). The predominant DRB1\*01 allele in most populations was \*0102. When DRB1\*15 and DRB1\*16 were grouped together as DR2 alleles, the genotype frequency in our population (16.5%) differed from that reported for the Alabama population and Ann Arbor populations ( $p = 0.03$  for both comparisons) but was similar to that re-

**TABLE 1** DRB1 and DQB1 allele frequency distribution in African-Americans from different regions of the United States

HLA-DRB <sup>a</sup>	SE US <i>n</i> = 112 % ( <i>n</i> ) <sup>b</sup>	NYC, Just <i>et al</i> , <i>n</i> = 242 % ( <i>n</i> )	11 <sup>th</sup> HCW, <i>n</i> = 132 % ( <i>n</i> )	Illinois, <i>n</i> = 86 % ( <i>n</i> )	Ann Arbor, <i>n</i> = 64 % ( <i>n</i> )	Dallas, <i>n</i> = 69 % ( <i>n</i> )	ASHI Workshop, <i>n</i> = 541 % ( <i>n</i> )	Alabama, <i>n</i> = 454 % ( <i>n</i> )
DRB1*01	4.5 (10)	5.4 (26)	5.3 (14)	4.1 (7)	1.56 (2)	0	6.77 (73)	8.52 (77)
*0101	30 (3)	31 (8)	35.7 (5)				26 (19)	
*0102	60 (6) <sup>c</sup>	69 (18)	64.3 (9)	14.2 (1)			70 (51)	
*0103	10 (1)	0	0	0			2.7 (2)	
*0104							2.7 (2)	11.24 (102)
DR2	16.5 (37)	18.5 (89)	15.9 (42)	13.5 (23)	25.7 (33)	12.73 (18)	15.64 (169)	
DRB1*15	13.8 (31)	16.6 (80)	13.2 (35)	12.3 (21)	21.8 (28)	11.29 (16)	13.87 (150)	
DRB1*16	2.7 (6)	1.9 (9)	2.7 (7)	1.2 (2)	3.9 (5)	1.44 (2)	1.77 (19)	
DRB1*03	12.5 (28)	13.4 (65)	14.2 (37)	16.97 (29)	13.28 (17)	10.7 (15)	13.22 (143)	14.3 (130)
*0301	6.25 (14)	6.80 (33)	7.0 (18)	ND	15.63 (10)	1.45 (1)	7.42 (80)	ND
*0302	4.90 (11)	6.60 (32)	7.20 (19)	1.16 (1)	7.81 (6)	13.04 (9)	5.80 (63)	ND
DRB1*04	4.9 (11)	4.6 (22)	3.8 (10)	2.33 (4)	3.9 (5)	5.1 (7)	5.96 (64)	4.7 (43)
*0401	18.2 (2) <sup>c</sup>	41 (9)	40 (4)			28.6 (2)	47 (30)	
*0403	9 (1)	0	0			0	0	
*0404	18.2 (2) <sup>c</sup>	18.2 (4)	10 (1)			0	7.8 (5)	
*0405	36.4 (4) <sup>c</sup>	31.8 (7)	20 (2)			28.6 (2)	37.5 (24)	
*0407	18.2 (2)	4.5 (1)	30 (3)			0	4.7 (3)	
*0411	0	4.5 (1)	0			14.2 (1)	3.1 (2)	
*04xx	0	0	0			28.6 (2)	0	
DR5	17.4 (39)	16.7 (81)	18.3 (48)	18.25 (31)	11.69 (15)	20.8 (29)	15.64 (170)	11.4 (104)
DRB1*11	12.0 (27)	12.4 (60)	13.3 (35)	15.2 (26)	8.56 (11)	15 (21)	12.25 (133)	
DRB1*12	5.4 (12)	4.3 (21)	5 (13)	3.05 (5)	3.13 (4)	5.8 (8)	3.39 (37)	
DR6	21.4 (48)	16 (77)	17.2 (45)	31.06 (53)	21.84 (28)	27.09 (38)	18.86 (204)	10 (91)
DRB1*13	20.5 (46)	13.7 (66)	15.7 (41)	29.9 (51)	21.06 (27)	24.89 (35)	17.25 (187)	
DRB1*14	0.9 (2)	2.3 (11)	1.5 (4)	1.16 (2)	0.78 (1)	2.2 (3)	1.61 (17)	
DRB1*07	8 (18)	12.2 (59)	9.8 (26)	3.65 (6)	13.22 (17)	7.2 (10)	10.48 (113)	8.8 (80)
DRB1*08	5.8 (13)	10.6 (51)	8 (21)	4.25 (7)	4.69 (6)	9.4 (13)	6.77 (73)	7.2 (65)
DRB1*09	6.7 (15)	0.6 (3)	2 (5)	0	3.13 (4)	3.6 (5)	5.00 (54)	1.26 (11)
DRB1*10	2.2 (5)	1.9 (9)	1.9 (5)	1.16 (2)	0	2.2 (3)	1.61 (17)	4.2 (38)
SE	9.8 (22)	11.4 (55)	9.8 (26)	ND	ND	5.1 (7)	13.5 (146)	

  

HLA-DQB1	<i>n</i> = 112 % ( <i>n</i> )	<i>n</i> = 241 % ( <i>n</i> )	<i>n</i> = 127 % ( <i>n</i> )	<i>n</i> = 57 % ( <i>n</i> )	ND	<i>n</i> = 69 % ( <i>n</i> )	<i>n</i> = 541 % ( <i>n</i> )	ND
DQB1*02	22.3 (50)	20.7 (100)	21.3 (54)	22.8 (26)		16.46 (23)	21.03 (227)	
DQB1*03	21.9 (49)	22 (106)	23.6 (60)	23.68 (27)		30.24 (42)	26.5 (287)	
*0301	73.5 (36)	74.5 (79)	82 (49)			64.3 (27)	74.9 (215)	
*0302	26.5 (13) <sup>f</sup>	19.8 (21)	13.0 (8)			9.5 (4)	16.7 (48)	
*0303		5.7 (6)	5 (3)			21.4 (9)	7.7 (22)	
*0305	0	0	0			0	0.7 (2)	
*03xx	0	0	0			4.8 (2)	0	
DQB1*04	4 (9)	6.6 (32)	10.2 (26)	7.9 (9)		5 (7)	7.83 (85)	
DQB1*05	22.3 (50)	19.5 (94)	20.5 (52)	15.8 (18)		15.56 (21)	20.06 (217)	
DQB1*06	29.5 (66)	31 (150)	23.3 (59)	28.9 (33)		30.4 (42)	24.60 (266)	

Resolution of alleles varied from high to low depending on the number of new alleles reported during the period of the study. For more details see Methods section.

<sup>a</sup> Individual allele frequencies for DRB1\*04\*, 03\*, and 01\* groups are listed as % of lower resolution parent. All other groups are listed as % of total N alleles.

<sup>b</sup> The *n* refers to the number of alleles, and % to *n*/2*x* the number of patients.

For Alabama % was derived by applying Bernstein's formula to the published phenotype frequency, and *n* was derived by multiplying % by the total number of alleles.

<sup>c</sup> In the Georgia study DRB1\*0102 could not be distinguished from \*0104; \*0401 from \*0408 and \*0416; \*0404 from \*0413; \*0405 from \*0409; and DQB1\*0302 from \*0303.

Abbreviations: ASHI = American Society for Histocompatibility and Immunogenetics; HCW = histocompatibility workshop; ND = not determined; NYC = New York City; SE = shared epitope; US = United States.

**TABLE 2** TNF1/TNF2 genotype and allele frequencies in healthy African-Americans residing in Georgia

TNF-308 genotype*	Genotype frequency, <i>n</i> = 59 <i>n</i> (%)
G/G	43 (72.9)
G/A	15 (25.4)
A/A	1 (1.7)

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TNF-308 allele	Allele frequency, <i>n</i> = 118 <i>n</i> (%)
G	101 (85.6)
A	17 (14.4)

\* TNF1 = G; TNF2 = A.

Abbreviation: TNF = tumor necrosis factor.

ported in the other populations. DRB1\*15 was the most common allele group in all populations, but the Ann Arbor study reported the highest frequency ( $p < 0.05$  compared with all studies except NYC). The frequency of DRB1\*16 was low in all populations ( $p =$  not significant [NS] between groups). The DRB1\*03 frequency was similar in all of the populations with the highest frequency in the Illinois population ( $p =$  NS for any comparison). Because DRB1\*0302 is a marker for sub-Saharan ancestry [31, 37, 38], we examined the frequency of 0301 and 0302 in the populations, and found no significant differences among populations with respect to these two alleles.

Although the DRB1\*04 frequencies were similarly low in all populations, allele frequency distributions differed when DRB1\*04 was typed to higher resolution. In our population, DRB1\*0405 was the most frequent allele and 0401 was less frequent; in the NYC and the IHW populations, \*0401 predominated and in the ASHI Workshop, \*0401 and \*0405 were almost equally prevalent [36]. In RA populations varying frequencies of the disease risk associated "shared epitope" containing alleles \*0401 and \*0405 have been reported. This may reflect differences in the background frequencies of these. For example DRB1 \*0405 is the most prevalent \*04 allele our AA RA patients from Georgia (JM McNicholl, unpublished observations).

No significant differences were seen in the frequency of the DR5 splits, DRB1\*11, and DRB1\*12 among the eight study populations. When DRB1\*11 and DRB1\*12 were grouped together into the DR5 group, the frequency of 17.4% found in our population was higher than the 11.4% frequency calculated for the Alabama population ( $p = 0.02$ ), but was similar to the frequency in other study populations.

In the DR6 splits, DRB1\*13 was the most frequent allele group in our population and was also frequent in other AA populations, although our population had a

frequency significantly lower than the Illinois ( $p = 0.03$ ) and significantly higher than the NYC populations ( $p = 0.02$ ). In all populations the frequency of DRB1\*14 was low. When DRB1\*13 and DRB1\*14 were grouped together into the DR6 group, the frequency of DR6 in our population (21.4%) was higher than the calculated genotype frequency of 10% for the Alabama population ( $p < 0.0001$ ), lower than the 31% ( $p = 0.03$ ) reported for the Illinois population, but not significantly different than the frequencies reported for the other study populations.

DRB1\*07 and DRB1\*08 frequencies were similar in frequency in all populations except for the significantly lower DRB1\*08 frequency in our population compared with NYC ( $p = 0.04$ ). The DRB1\*10 frequency was similarly low in all populations.

The largest disparity in HLA frequencies between our population and the other populations was for DRB1\*09. Our population had a significantly higher frequency (6.7%) than reported in the NYC, IHW, Illinois, and Alabama populations (all  $< 2\%$ ;  $p$  values of  $< 0.0001$ , 0.008, 0.0005, and  $< 0.0001$ , respectively compared with our population), and higher (but not significantly) than the frequencies reported in the remaining studies as well as the frequency reported in 30 healthy African Columbians from Quibdo, Columbia (2%) [39]. High frequencies of DRB1\*09 have been also been noted in the Gambia, West Africa (7.1%), and in Malawi, South-Central Africa (10.4%) [40].

#### Comparisons With Five Other AA Populations at the DQB1 Locus

Our population and all the populations were similar in the frequency of DQB1\*02,03, 05, and 06 allele groups. DQB1 \*06 predominated in our population and in the NYC and Illinois populations whereas DQB1\*03 predominated in the ASHI workshop study; in the IHW and Dallas studies these alleles had equal frequencies. Where typing was carried out to higher resolution, DQB1\*0301 was the most frequent \*03 allele, with high frequencies in all populations. The least predominant group was DQB1\*04 and a significant difference in allele frequency was found for DQB1\*04, which was lowest in our population ( $p = 0.009$  vs the IHW population frequency of 10.2%). Resolution of alleles varied from high to low depending on the number of new alleles reported during the period of the study.

The two most frequent likely haplotypes in our study were DRB1\*1503(01/04)/DQB1\*0602(03) and DRB1\*0701(02)/DQB1\*0201(02) (7.5% and 7.9%, respectively). Although the resolution could not exclude that the alleles in parentheses were part of the haplotype, comparison with known haplotypic assignment in the ASHI Workshop study where the two most common

DR/DQ haplotypes were DRB1\*1503/DQB1\*0602 and DRB1\*0701/DQB1\*0201 (with frequencies of 7.0% and 6.7%, respectively), suggests that the first allele assignment in the above haplotypes are the most prevalent.

### TNF $\alpha$ -308 Promoter Polymorphism

Limited data are available on the frequency of TNF $\alpha$  promoter polymorphisms among AAs. The 14.4% frequency of the TNF $\alpha$  -308A allele in our AA population is higher, but not significantly ( $p = 0.09$ ), than the 7.8% frequency reported in 64 AA controls recruited for a study of SLE patients in Baltimore [41] but similar to the 13% frequency reported among 15 individuals of AA and African-Caribbean descent living in Boston [42]. The frequency in our population is also similar to frequencies reported in Africans from the Gambia (16.0% and 17%) [43, 44], Malawi (12% and 11%) [42, 44], and Nigeria (13%) [42], and higher, but not significantly, than that reported from Tanzania (9%) [45]. Larger groups of AAs would have to be sampled from different US regions to determine whether there are regional differences in the distribution of this allele among US AAs. However, because of its linkage to the HLA locus, such differences are likely to exist.

### Regional Diversity in the Major Histocompatibility Complex in AAs

Our data and previous publications indicate the regional differences in major histocompatibility complex gene distribution among AAs in the US. Our HLA data are most similar to the ASHI Workshop data. In that study, geographical differences in the distribution of DR2, 3, 5, and 6 antigens were noted within UNOS (United Network for Organ Sharing) regions 2, 3, 4, 7, and 11 in the United States. Because UNOS region 3 (Florida, Alabama, Georgia, and Mississippi) overlaps our population sample, we compared both studies using gene frequencies for UNOS 3 estimated from a sample size of 50 [36]. Significant differences exist for DR2 and DR5 between UNOS region 3 and our population (5%,  $p = 0.004$  and 8.4%,  $p = 0.03$  for comparisons with our data). Interestingly, our population, of whom 54% are Georgia residents, differs most from the Alabama population which reported a higher frequency of DR1 ( $p = 0.04$ ) and lower frequency of DR2 ( $p = 0.03$ ), DR5 ( $p = 0.02$ ), DR6 ( $p < 0.0001$ ), and of DR9 ( $p < 0.0001$ ) [19]. Because the three southeastern US studies had varying sample sizes, typing methodologies, and recruitment strategies, another study using similar sampling and HLA typing methods could confirm any true regional differences in this area. However, genetic disparities between two geographically adjacent populations can exist. For example, even in AAs from Alabama, significant

gene frequency differences have been found for several alleles of two variable nucleotide tandem repeat loci in AAs from southern compared to northern Alabama counties [19].

Our HLA and TNF data extend findings from previous studies demonstrating regional differences in the distribution of major histocompatibility complex polymorphism in AAs. These findings may be due to different ancestral origins, migration and admixture, and may be important to consider when constructing organ donor pools. For example local, rather than national searches for matched donors might be the most successful. These data also have implications for African-American populations when performing disease association studies, understanding vaccine responses, and for studies of genetic diversity and evolution.

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