Gene Encoding Duffy Antigen/Receptor for Chemokines Is Associated with Asthma and IgE in Three Populations

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Rationale: Asthma prevalence and severity are high among underserved minorities, including those of African descent. The Duffy antigen/receptor for chemokines is the receptor for *Plasmodium vivax* on erythrocytes and functions as a chemokine-clearing receptor. Unlike European populations, decreased expression of the receptor on erythrocytes is common among populations of African descent, and results from a functional T-46C polymorphism (rs2814778) in the promoter. This variant provides an evolutionary advantage in malaria-endemic regions, because Duffy antigen/receptor for chemokines-negative erythrocytes are more resistant to infection by *P. vivax*.

Objectives: To determine the role of the rs2814778 polymorphism in asthma and atopy as measured by total serum IgE levels among four populations of African descent (African Caribbean, African American, Brazilian, and Colombian) and a European American population.

Methods: Family-based association tests were performed in each of the five populations to test for association between the rs2814778 polymorphism and asthma or total IgE concentration.

Measurements and Main Results: Asthma was significantly associated with the rs2814778 polymorphism in the African Caribbean, Colombian, and Brazilian families ($P < 0.05$). High total IgE levels were associated with this variant in African Caribbean and Colombian families ($P < 0.05$). The variant allele was not polymorphic among European Americans.

Conclusions: Susceptibility to asthma and atopy among certain populations of African descent is influenced by a functional polymorphism in the gene encoding Duffy antigen/receptor for chemokines. This genetic variant, which confers resistance to malarial parasitic infection, may also partially explain ethnic differences in morbidity of asthma.

Keywords: Duffy antigen/receptor for chemokines; continental population groups; lung diseases; hypersensitivity

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The prevalence and severity of allergic airway diseases is especially high among individuals of African descent, even after adjusting for demographic and socioeconomic factors. Although it is unknown to what extent genetic susceptibility contributes to disparities in risk for atopic asthma, both linkage and association studies have shown evidence of genetic differences among ethnic and racial groups.

What This Study Adds to the Field

In this study we demonstrate that a genetic variant that determines the lack of expression of the Duffy antigen/receptor for chemokines in African ancestry populations can explain, at least in part, the observed disparities across ethnic groups.

The prevalence and severity of allergic airway diseases is especially high among individuals of African descent, even after adjusting for demographic and socioeconomic factors (1, 2). Higher total IgE levels have also been observed in individuals of African descent compared with individuals of European descent (3, 4). Although it is unknown to what extent genetic susceptibility contributes to disparities in risk for atopic asthma, both linkage and association studies have shown evidence of genetic differences among ethnic and racial groups (4–7).

The Duffy antigen/receptor for chemokines (DARC) is expressed on red blood cells (RBCs), where it acts as the receptor for malarial parasites *Plasmodium vivax* and *P. knowlesi* (which infects primarily primates); it is also expressed on endothelial cells of postcapillary venules, Purkinje cells of the cerebellum, kidney epithelial cells lining collection tubules, and type I pneumocytes of the lung (8–11). A point mutation in a consensus binding site of the erythroid transcription factor GATA1 in the DARC promoter (T-46C) selectively decreases DARC expression on RBCs with a gene dosage effect (12). This underlies the molecular basis for the DARC-negative phenotype (13) observed in most sub-Saharan and West African populations (14). This phenotype, also named Duffy negative and Duffy null, is largely absent or occurs at a low frequency in other ethnic groups (15) and is determined by the CC genotype. The fixation of the C allele (FY*0) in African populations has conferred a selective advantage in malaria-endemic regions because DARC-negative erythrocytes are resistant to infection by *P. vivax* (14, 16).

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DARC has also been described as a chemokine “sink” (17) to the extent that it binds chemokines of both the CXC and CC groups (8), some of which are persistently upregulated in asthmatic airways especially after viral infections (18). An exaggerated inflammatory response observed in the lungs and liver of *DARC* knockout mice is consistent with the idea that the molecule functions as a chemokine-clearing receptor (19). It has been suggested that *DARC* may limit the actions of chemokines and alter allergic inflammation (20, 21).

Because it has a striking difference in allelic frequency at the *DARC* T-46C polymorphism (8) between African-descended populations and populations of European ancestry (22), this single-nucleotide polymorphism has been extensively used as an “ancestry informative marker” in population genetic studies to estimate ancestry proportions in admixed populations (15). Given the crucial functional role of the CC genotype in abolishing expression of this chemokine-clearing receptor on RBCs, with potential implications in inflammatory diseases such as asthma, and the higher frequency of the C variant among individuals of African descent, we hypothesized that the *DARC* T-46C polymorphism may be associated with a higher allergic diathesis reflected by a positive association between CC genotype and allergic airway diseases or IgE response.

METHODS

Subjects

Nuclear and extended families belonging to five ethnically distinct populations (described in the following sections) were studied. Blood samples were collected from all subjects for serum measurements of total serum IgE (tIgE) concentrations and DNA extraction for genotyping. Asthma definition and tIgE measurements were previously described (23–26) for each population as explained in detail in the online supplement.

African Caribbeans. Asthma probands and their family members (745 individuals from 125 pedigrees) from Barbados who self-reported as African Caribbean were recruited as previously described (23). All subjects gave verbal and written consent as approved by the Johns Hopkins Institutional Review Board and the Barbados Ministry of Health.

African Americans and European Americans. African American and European American probands and their family members (368 individuals in 99 families and 493 subjects in 122 families, respectively) were ascertained as part of the Collaborative Study on the Genetics of Asthma (4, 7) in the Baltimore–Washington, DC metropolitan area in a study previously approved by the Johns Hopkins and Howard University Institutional Review Boards (4).

Colombians. Colombian probands and their family members who attended one of three public health centers in Cartagena and their family members were included (655 individuals in 167 families). A full verbal explanation of the investigation was provided and all subjects gave written consent as previously approved by the Bioethics Committee of the School of Medicine of the University of Cartagena (Cartagena, Colombia).

Brazilians. Seven hundred and forty-nine subjects comprising 82 nuclear or extended families living in five villages in the Conde district of Bahia, Brazil, recruited according to a whole population ascertainment scheme, and who had an available DNA sample for genotyping and complete phenotype data, were included in this analysis. In this population, individuals with asthma were identified on the basis of a modified ISAAC questionnaire (27, 28). The research protocol was approved by the institutional review boards of the Johns Hopkins University School of Medicine and of the Federal University of Bahia and was endorsed by the National Commission for Ethics in Human Research in Brazil.

Genotyping

DNA was extracted by standard protocols. Genotyping of the *DARC* T-46C polymorphism was performed with either the TaqMan probe-based, 5′ nuclease allelic discrimination assay on a 7900HT sequence

detection system (Applied Biosystems, Foster City, CA) or by polymerase chain reaction with sequence-specific oligonucleotides as previously described (22) and in the online supplement.

Statistical Methods

Primary outcome variables for the family-based association analyses of each of the five populations were asthma status as a dichotomous trait (i.e., presence/absence) and log-transformed, age- and sex-adjusted tIgE levels (log[tIgE]). Clinical characteristics of each study population including means and SDs for continuous measures and *t*-test *P* values comparing means among populations, proportions for binary traits, and analysis of variance test determining the association between *DARC* T-46C genotypes and tIgE in subjects with asthma from each population were calculated with STATA 8.2 (StataCorp LP, College Station, TX). In each family data set, tests of linkage and association between the marker and asthma and log[tIgE] were conducted using the family-based association test (FBAT version 2.0.2C; available at <http://www.biostat.harvard.edu/~fbat/default.html>). A *P* value less than 0.05 was considered statistically significant.

RESULTS

Demographic Characteristics

Clinical and demographic characteristics of founders and all individuals in each population are presented in Table 1. Founders were older than the total population, as is to be expected. The average age ($\pm$ SD) of offspring with asthma was 21.8 ± 13.23 years in the African Caribbean families, 14.6 ± 7.7 years in the African American families, 20.0 ± 10.8 years in the European American families, 23.27 ± 17.03 years in the Brazilian families, and 17.54 ± 10.1 years in the Colombian families. Among African Caribbean, African American, and European American family members (745, 368, and 494 individuals, respectively), means of tIgE concentrations were similar to those described previously (23, 29, 30). In general, tIgE levels were significantly different between founders in each pair of populations except for the comparison of African American and European American populations ($P = 0.161$). Brazilians showed the highest IgE levels among the five populations, which can be explained by the high rate (83.5%) of extracellular parasitic disease in this Brazil population (31), and the lowest levels were observed in the European American population. Total IgE was significantly

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF AFRICAN CARIBBEAN, AFRICAN AMERICAN, BRAZILIAN, COLOMBIAN, AND EUROPEAN AMERICAN POPULATIONS

Population	Characteristic				
	No. of Subjects	Percentage Male	Age (yr)*	Percentage Asthmatic	tIgE*†
African Caribbean					
Founders	263	53.2	46.57 $\pm$ 10.93	9.4	2.78 $\pm$ 0.67
Total	745	50.0	29.29 $\pm$ 16.86	39.1	2.79 $\pm$ 0.67
African American					
Founders	162	43.5	43.01 $\pm$ 9.81	35.0	2.33 $\pm$ 0.64
Total	368	48.3	27.97 $\pm$ 16.46	69.5	2.57 $\pm$ 0.66
Brazilian					
Founders	129	38.7	43.95 $\pm$ 19.94	28.2	3.42 $\pm$ 0.52
Total	749	44.0	27.26 $\pm$ 18.77	29.9	3.40 $\pm$ 0.47
Colombian					
Founders	289	50.5	45.9 $\pm$ 12.36	15.7	2.95 $\pm$ 0.55
Total	655	49.6	35.50 $\pm$ 18.01	41.4	3.03 $\pm$ 0.56
European American					
Founders	242	51	48.43 $\pm$ 12.51	16.2	2.20 $\pm$ 0.72
Total	494	47	35.09 $\pm$ 18.44	57.4	2.19 $\pm$ 0.74

* Values represent means $\pm$ SD.

† Log-transformed total IgE (tIgE) concentrations are represented as nanograms per milliliter, adjusted for age and sex.

higher in subjects with asthma compared with those without asthma among the African Caribbean ($P = 6.6 \times 10^{-12}$), African American ($P = 0.015$), Colombian ($P = 0.003$), and European American ($P = 0.0001$) families, but not in the Brazilian families ($P = 0.85$). The percentage of subjects with asthma in the total population ranged from 29.9 to 69.5% across these five populations. The lowest percentage was observed in the Brazilian population and the highest in African American families, as summarized in Table 1.

Allelic and Genotypic Frequencies across Five Ethnically Distinct Populations

DARC T-46C genotypes were in Hardy-Weinberg equilibrium among founders from all five populations (see Table E1 in the online supplement). Frequencies of the C variant and its genotypes were significantly different across the five study groups (Figure 1A, and see Table E1). The C allele occurred at the highest frequency (86.7%) in the 263 African Caribbean founders compared with a low of 0.01% (5 copies) in the 242 European American founders. Frequency of the C allele in the European American population was significantly lower compared with each of the four African ancestry populations ($P < 0.0001$ for each comparison; see Table E1). Similar trends were observed with genotype frequencies, which varied significantly across the five populations ($P < 0.001$ for each pair of comparisons). The CC genotype (which determines the lack of expression of DARC in RBCs) was observed in 0.4% (1 individual) among the European Americans, and the frequency increased progressively through Colombians (10.7%), Brazilians (31.0%), African Americans (58.0%), and African Caribbeans (77.1%, or 203 of 263 subjects; Figure 1B).

Figure 1. Gene encoding Duffy antigen/receptor for chemokines (*DARC*) T-46C allele and genotype frequencies among African Caribbean, African American, Brazilian, Colombian, and European American founders.

Association Analyses of the *DARC* T-46C Polymorphism

A significantly higher transmission of the *DARC* CC genotype to offspring with asthma than would be expected by chance was observed in African Caribbean and Brazilian families ($P = 0.042$ and $P = 0.050$, respectively; Table 2). The CT genotype was significantly undertransmitted in both African Caribbean and Colombian families ($P = 0.015$ and $P = 0.021$, respectively; see Table 2).

Significant associations were also observed between tIgE levels and the T-46C polymorphism in African Caribbean families (CC genotype, $P = 0.045$; CT genotype, $P = 0.028$) and Colombian families (CC genotype, $P = 0.05$; CT genotype, $P = 0.007$; see Table 2). No association was observed between asthma or tIgE levels and the CC genotype in African American families. The CC genotype was significantly associated with higher tIgE levels when compared with the combined CT and TT genotypes in a subset of unrelated subjects with asthma in all three populations (African American, $P = 0.028$; Colombian, $P = 0.027$; and Brazilian, $P = 0.017$; Figure 2). Because the majority of European American families (95%) were uninformative for this marker, association tests were not performed in this group.

DISCUSSION

In this study we provide, for the first time, consistent evidence of association of the *DARC* T-46C polymorphism with both asthma and tIgE in African Caribbean and Colombian families, with asthma in Brazilian families, and with tIgE in a subgroup of

TABLE 2. FAMILY-BASED ASSOCIATION TEST RESULTS FOR *DARC* T-46C POLYMORPHISM AND ASTHMA AND TOTAL IgE LEVELS IN FOUR POPULATIONS OF AFRICAN DESCENT

Study Population	DARC T-46C Genotype	No. of Informative Families	Z Score	FBAT P Value	
African Caribbean	Asthma	CC	42	2.025	0.042
		CT	45	-2.415	0.015
		TT	8	1.214	0.224
	Total serum IgE	CC	58	2.001	0.045
		CT	62	-2.201	0.028
		TT	9	0.790	0.429
African American	Asthma	CC	35	-0.073	0.941
		CT	40	0.394	0.693
		TT	15	-0.593	0.552
	Total serum IgE	CC	23	0.626	0.531
		CT	26	-0.274	0.784
		TT	11	-0.501	0.616
Brazilian	Asthma	CC	35	1.922	0.050
		CT	57	-1.435	0.151
		TT	35	-0.069	0.944
	Total serum IgE	CC	48	0.803	0.421
		CT	77	-1.096	0.273
		TT	45	-0.672	0.501
Colombian	Asthma	CC	43	1.242	0.214
		CT	83	-2.300	0.021
		TT	73	1.709	0.087
	Total serum IgE	CC	43	1.911	0.050
		CT	82	-2.687	0.007
		TT	72	1.699	0.089

Definition of abbreviations: *DARC*, gene encoding Duffy antigen/receptor for chemokines; FBAT, family-based association test.

Figure 2. Comparison of total IgE levels between carriers of the CC genotype and carriers of the combined CC and CT genotypes among unrelated subjects with asthma within (A) African Caribbean, (B) African American, (C) Colombian, and (D) Brazilian groups. No comparisons were performed among the European Americans because of the low frequency of the CC genotype (0.4%) in this population. The line in the middle of the box represents the median and the lines that form the box correspond to the 25th and 75th percentiles.

unrelated subjects with asthma from African American, Brazilian, and Colombian populations. This study therefore supports the notion that certain genetic factors may render individuals of African ancestry more susceptible to develop an allergic response and asthma, which may partially explain the disproportionately higher morbidity and mortality for asthma compared with populations of European descent.

In contrast to the Brazilian, African Caribbean, and Colombian families, we found no evidence of linkage or association between the *DARC* T-46C polymorphism with either asthma or tIgE levels in African American families, possibly because of differences in statistical power and sample size. However, there was a significant association between the CC genotype and high tIgE levels among African American subjects with asthma, supporting the association between the *DARC* T-46C polymorphism and tIgE in this group. The demographic history of these four populations is similar, that is, they each share common West African ancestors resulting from the African slave trade; however, each of these four populations had a different dynamic of admixture with Europeans and native Americans (15, 32–34) and they currently reside in diverse environmental settings. Although African Americans in general, and individuals from Baltimore and Washington, DC in particular, have a high African ancestry proportion (15, 35, 36) compared with Colombians (C. Vergara, L. Caraballo, D. Mercado, S. Jimenez, W. Rojas, N. Rafaels, T. Hand, M. Campbell, Y. Tsai, L. Gao, C. Duque, S. Lopez, G. Bedoya, A. Ruiz-Linares, and K.C. Barnes, unpublished data) (32) and Brazilians (33, 37), specific environmental factors and potential gene–environment interactions are likely to be considerably different between African American and the other tropical populations, which could account for differences in the results observed here. The significantly lower mean total serum IgE levels among the African American group (2.57 ± 0.66) compared with African Caribbeans (2.79 ± 0.67), Colombians ($2.95 \pm$

0.55), and Brazilians (3.42 ± 0.52 ; see Table 1), all of which were based in the tropics, could suggest less severe allergic diathesis in that group. It might also suggest the impact of environmental factors present in the tropics but not present in the U.S. inner city, temperate region where the African American group resides. Alternatively, the lack of association of T-46C polymorphism with tIgE levels observed among the Brazilian families compared with other participants in this study may be due to stronger environmental stimulus for IgE production because this population resides in a region currently endemic for schistosomiasis, with prevalence ranging from 13.7 to 88.9% across the five villages comprising this group (31). Analysis of short tandem repeats around the *DARC* gene showed that linkage disequilibrium extends across a 30-cM region, but is strongest for a flanking interval of 5–10 cM centered around the T-46C polymorphism in African Americans (38). Although our findings could be the result of an indirect association with a variant(s) in this highly conserved linkage disequilibrium block, animal and human studies focusing on the functional relevance of this polymorphism support the notion that the C variant itself increases the risk of asthma and atopy. *DARC* facilitates uptake of chemokines into cells but does not itself transmit a signal because it lacks a motif in the second intracellular loop associated with G-protein coupling (39). In studies of *DARC*-negative RBCs (17), cells transfected with cloned *DARC* (39) and mice with lipopolysaccharide-induced endotoxemia (in which the *DARC* gene has been deleted) indicate the receptor functions as a clearance receptor for chemokines in blood and tissue, with both antiinflammatory and antiangiogenic roles. CC and CXC chemokines are potent chemoattractants for eosinophils, basophils, monocytes, and T cell subsets and are believed to be crucial mediators of inflammation in asthma (40, 41). *DARC* has previously been associated with the inflammatory process in prostate cancer (42, 43), pre-eclampsia in women from West Africa (44), and bone mineral

density in a murine model (45). Similar to asthma, these clinical conditions exhibit a different prevalence between African Americans and individuals of European descent (46, 47), which may also be explained by variations in the exposure stimulating chemokines and/or differences in the function of DARC among these populations. The alternative direction of the association in our family-based analyses between the CC genotype (which confers risk in African Caribbeans and Brazilians) and CT genotype (as a “protector” genotype in African Caribbeans and Colombians) with asthma as well as tIgE suggests subjects with asthma carrying the CC genotype (DARC-negative) may be more severely affected because of longer or greater exposure to inflammatory chemokines compared with DARC-positive subjects (48). Unfortunately, the limited number of informative families for the TT genotype negates the estimation of any association with this genotype in this study. We believe that this factor also influenced the lack of association between the CC genotype and asthma in Colombians because of the low frequency of the C allele in this population.

The DARC T-46C CC genotype was reported as conferring a significantly higher risk for acquiring HIV infection in African Americans (49). Other numerous examples of ethnic differences in frequencies of variants associated with inflammatory and infectious disease abound, including the $\Delta 32$ CCR5 genotype, which protects against macrophage-tropic HIV infection in African populations (50); a polymorphism in the *RANTES* promoter that significantly increases transcription of the *RANTES* gene in T lymphoid and mast cells (48); and a comprehensive analysis of differences in allelic distribution of single-nucleotide polymorphisms correlated with differential gene expression for several cytokine genes (e.g., genes encoding IL-6, IL-10, tumor necrosis factor- α , IFN- γ , and transforming growth factor- β_1) in Caucasian, Canadian aboriginal, and Filipino cohorts (51). As described for DARC T-46C in Africans and malaria, the evolution of this unique cytokine genotype profile may be linked to aboriginal adaptation to selection pressures related to an environment in which helminthic, parasitic, and fungal infections predominated.

In summary, we provide novel evidence of ethnic-specific susceptibility to allergic airway disease based on a specific genotype. Our findings require further investigation among other ethnic groups but may provide, at least in part, an explanation for the higher asthma morbidity observed in populations of African descent compared with other ethnic groups. Once the genetic basis of this disparity is better understood, it may facilitate the design of specific therapies targeting these molecules in a population-specific way.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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