Sequence, Haplotype, and Association Analysis of *ADR*β*2* in a Multiethnic Asthma Case-Control Study

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Rationale: **The comprehensive evaluation of gene variation, haplotype structure, and linkage disequilibrium is important in understanding the function of β_2-adrenergic receptor gene (*ADR*β*2*) on disease susceptibility, pulmonary function, and therapeutic responses in different ethnic groups with asthma.**

Objectives: **To identify *ADR*β*2* polymorphisms and haplotype structure in white and African American subjects and to test for genotype and haplotype association with asthma phenotypes.**

Methods: **A 5.3-kb region of *ADR*β*2* was resequenced in 669 individuals from 429 whites and 240 African Americans. A total of 12 polymorphisms, representing an optimal haplotype tagging set, were genotyped in whites (338 patients and 326 control subjects) and African Americans (222 patients and 299 control subjects).**

Results: **A total of 49 polymorphisms were identified, 21 of which are novel; 31 polymorphisms (frequency > 0.03) were used to identify 24 haplotypes (frequency > 0.01) and assess linkage disequilibrium. Association with ratio $(FEV_1/FVC)^2$ for single-nucleotide polymorphism +79 ($p < 0.05$) was observed in African Americans. Significant haplotype association for $(FEV_1/FVC)^2$ was also observed in African Americans.**

Conclusions: **There are additional genetic variants besides +46 ($Gly^{16}Arg$) that are important in determining asthma phenotypes. These data suggest that the length of a poly-C repeat (+1269) in the 3′ untranslated region of *ADR*β*2* may influence lung function, and may be important in delineating variation in β-agonist responses, especially in African Americans.**

Keywords: asthma; β_2-adrenergic receptor; β-agonist therapy; DNA polymorphisms; pharmacogenomics

β-Agonists are the most common bronchodilators used to treat airflow limitation associated with obstructive airways diseases. Individual therapeutic responses to β-agonists differ, and can be influenced by several factors, including degree of baseline airway obstruction, ethnicity, and age (1, 2). Therapeutic responses can also be affected by receptor desensitization produced by prolonged β-agonist use during regular therapy (3, 4). The mechanisms responsible for heterogeneity in β-agonist responsiveness have not been completely elucidated; however, there is evidence that genetic variation in the β_2-adrenergic receptor gene (*ADR*β*2*) may be one important factor (5).

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Gene variation and haplotypes relating to the effects of the gene *ADR*β*2* on disease susceptibility and therapeutic responses had been previously studied in only a limited fashion.

What This Study Adds to the Field

There are additional *ADR*β*2* genetic variants besides +46 ($Gly^{16}Arg$) and +79 ($Gln^{27}Glu$) that are important in determining asthma phenotypes.

The *ADR*β*2* is located on chromosome 5q31 (6), a region that is genetically linked to asthma and related phenotypes (7, 8). Reihaus and coworkers (9) described nine coding polymorphisms in *ADR*β*2*, four of which ($Gly^{16}Arg$, $Gln^{27}Glu$, $Val^{34}Met$, and $Thr^{164}Ile$) create nonsynonymous changes in the amino acid sequence. *In vitro* studies by Green and coworkers (10) showed that β_2-adrenergic receptors containing Gly^{16}/Gln^{27} and Gly^{16}/Glu^{27} had greater down-regulation after isoproterenol exposure than did receptors containing Arg^{16}/Gln^{27}, whereas Arg^{16}/Glu^{27} receptors exhibited no down-regulation. Several association studies have found that the Gly^{16} allele correlated with more severe asthma (11, 12), characterized by corticosteroid use and nocturnal symptoms (13–15), whereas subjects homozygous for Arg^{16} had lower lung function (16). In contrast, analysis of $Gln^{27}Glu$ found that the Glu^{27} allele was associated with a decrease in airway responses (17–19), whereas Gly^{16} was associated with asthma susceptibility (12, 20, 21). Other studies have not replicated these findings (22–24). Recent meta-analyses have reported inconsistent results that exist between different *ADR*β*2* association studies (25, 26).

Current interest in *ADR*β*2* has focused on the role of $Gly^{16}Arg$ and $Gln^{27}Glu$ variations in modulating responses to β-agonist treatment. Martinez and coworkers (27) found that children homozygous for Arg^{16} were 5.3 times more likely to show reversibility to albuterol (> 15.3% of predicted FEV_1) than were homozygous Gly^{16} subjects. Heterozygous Gly^{16}/Arg^{16} children showed intermediate responses to albuterol, whereas variations at +79 ($Gln^{27}Glu$) had no significant effect. A reduced response to β-agonist therapy in homozygous Gly^{16} subjects has been replicated in other studies (28, 29). However, recent reports have shown adverse effects to treatment with β-agonists in Arg^{16} homozygous patients with asthma. In an ACRN (Asthma Clinical Research Network; National Heart, Lung, and Blood Institute) study (30), subjects with asthma homozygous for Arg^{16} treated with regular, short-acting β-agonist experienced significant declines in A.M. and P.M. PEF compared with Gly^{16} homozygous subjects.

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Taylor and coworkers observed similar effects, as well as increased exacerbations in Arg^{16} homozygous subjects on regular albuterol treatment, but did not report changes in Arg/Arg subjects receiving intermittent albuterol or the long-acting β-agonist salmeterol (31). Subsequently, in a genotype-stratified, placebo-controlled, double-blind ACRN study (BARGE [β-Agonist Response by Genotype]) (32), Arg^{16} homozygous subjects with asthma receiving regular, short-acting β-agonist therapy had lower peak flow rates and increased symptoms compared with Arg^{16}/Arg^{16} subjects treated with intermittent, short-acting anticholinergics (ipratropium). In contrast, Gly^{16}/Gly^{16} subjects receiving regular albuterol experienced improvement in peak flow rates and fewer daily asthma symptoms. A recent report on the responses to salmeterol in two ACRN trials (33, 34) found that Arg^{16}/Arg^{16} subjects had decreased responses to salmeterol in the presence or absence of concurrent inhaled corticosteroids (35).

Genetic variations other than $Gly^{16}Arg$ and $Gln^{27}Glu$ may also be important (36). In a family-based association study by Silverman and coworkers (37), polymorphisms −654 and +46 ($Gly^{16}Arg$) were associated with post-bronchodilator FEV_1, whereas the polymorphism +523 ($Arg^{175}Arg$) was associated with bronchodilator response. Drysdale and coworkers (38) described the molecular haplotypic structure of *ADRβ2* in several ethnic groups and reported that haplotypes 2, 4, and 6 comprised 94.5% of the haplotypic variation in whites and haplotypes (1, 2, 4, and 6) comprised 92.3% of the haplotypic variation in African Americans. Although they also reported an affect of haplotype pairs on acute bronchodilator responses to albuterol, a recent report by Taylor and colleagues in 176 subjects with asthma reported no significant relationship between haplotype pairs and bronchodilator response (38).

The primary objective of this study was to comprehensively sequence *ADRβ2* to thoroughly evaluate gene variation, linkage disequilibrium (LD), and haplotype structure. Because of recent evidence concerning rare adverse effects of β-agonist therapy in African Americans (39), we sequenced *ADRβ2* in a large sample of African Americans as well as whites (n = 669). Because these adverse events are infrequent, identification of rare *ADRβ2* variants could identify genetic variants or haplotypes that may be important in these adverse therapeutic responses. A secondary objective was to determine whether *ADRβ2* genetic variants and/or haplotypes were associated with asthma phenotypes, specifically pulmonary function, in a well-characterized, multiethnic case-control population.

METHODS

Study Populations

Asthma cases consisted of unrelated subjects with either bronchial hyperresponsiveness (BHR) or bronchodilator reversibility (if $FEV_1 \leq$ 60% predicted), less than 3 pack-years of tobacco exposure, appropriate asthma symptoms, and a physician's diagnosis of asthma (40). Control subjects had no personal or family history of asthma (46, 47). These genetic studies were approved by the institutional review board at the University of Maryland and Wake Forest University School of Medicine, and informed consent was obtained from all subjects (41, 46, 47).

The second population represented a subset of the Childhood Asthma Management Program (CAMP), which was a multicenter randomized, double-blinded clinical trial testing the safety and efficacy of inhaled corticosteroids compared with placebo and nedocromil. Trial design and methodology have previously been published (41–43). Children enrolled in CAMP were 5–12 yr of age, with mild to moderate persistent asthma that was characterized by asthma symptoms, medication use, and BHR. The CAMP Genetics Ancillary Study was approved by each study center's institutional review board, and informed consent/assent was obtained from all participants and their parents.

DNA sequencing was performed using a subset of samples from the case and control population (283 white and 180 African American) (44) and the CAMP study population (136 white and 60 African American) (37). Case-control association studies were performed on the adult asthma case-control subjects (45).

DNA Sequencing and Genotyping of *ADRβ2* Polymorphisms

The strategy and methods for generating and sequencing polymerase chain reaction products have been previously described (46). A 10,000-bp genomic reference sequence of *ADRβ2* was ascertained from the University of California Santa Cruz Genomic Browser website (available online at http://genome.ucsc.edu/) and aligned with the mRNA reference sequence M15169 (GenBank reference no.). A region −3,470 bp 5′ of the ATG start site to +1,886 bp after the ATG start site was sequenced. DNA sequence data were aligned and polymorphisms identified using Sequencer DNA analysis software (Gene Codes Corporation, Ann Arbor, MI). Genotyping was performed using the MassARRAY genotyping system (Sequenom, Inc., San Diego, CA). CEPH (Centre d'Etude du Polymorphisme Humain) controls and blanks were included for quality control and error checking. The 3′ untranslated region (UTR) poly-C polymorphism was genotyped by fragment analysis on an ABI 3700 DNA Analyzer (Applied Biosystems, Foster City, CA).

Statistical Methods

Hardy-Weinberg equilibrium testing was performed on genotyped polymorphisms using the GDA computer programs (47) and SAS/Genetics, 2002 (SAS Institute, Cary, NC). LD, in terms of D′ and r^2, were calculated using the program Haploview (http://www.broad.mit.edu/mpg/haploview/index.php) (48). *t* Tests and analyses of variance were performed for quantitative measurements of pulmonary function (% predicted FEV_1, % predicted FVC, $[FEV_1/FVC]^2$ [ratio squared was used for normality], BHR [log of provocative concentration of methacholine causing a 20% drop in FEV_1 (PC_{20})], bronchodilator reversibility, and post-bronchodilator FEV_1) to test for association using the case and control populations. Association tests between haplotypes and asthma phenotypes were performed using Haplo.Score (http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm) (49).

RESULTS

Case-Control Population

Subjects with asthma tended to be younger than the control subjects with higher IgE levels, and 90% had positive allergy skin test (*see* Table E1 in the online supplement). Percent-predicted FEV_1 was slightly lower in the African-American subjects, but both ethnic groups had similar FEV_1/FVC ratios and levels of BHR. Clinical data for the CAMP participants have been previously published (41, 42, 50).

Sequence Analysis Shows 49 Variants in *ADRβ2*

ADRβ2 polymorphisms were identified according to base pair numbering described by Drysdale and colleagues (38), in which the +1 position represents the first base in the ATG start codon. A total of 49 polymorphisms were identified (Figure 1 and Table 1), of which 21 were novel—1 of which was coding (+66; $His^{22}His$). Polymorphism −709, identified by Drysdale and coworkers (38), was not detected. All novel polymorphisms had a minor allele frequency less than 10% in all ethnic groups, with the exception of −3159 (≥ 15% in all ethnic groups). Allele frequencies of $Gly^{16}Arg$, $Gln^{27}Glu$, and $Thr^{164}Ile$ were similar to those reported for whites and African Americans (38, 51). A rare nonsynonymous polymorphism, $Ser^{220}Cys$, was found in African Americans (allele frequency = 0.03), but was not detected in whites. Two novel insertion/deletions (InDels; −2051 and −376) were identified in African Americans. InDel −2051 consists of a 4 or 5 poly-C repeats, whereas InDel −376 consists of a 25-base duplication. Sequencing of the 3′ UTR revealed a poly-C repeat starting 23 bases after the TAA stop and varying in size

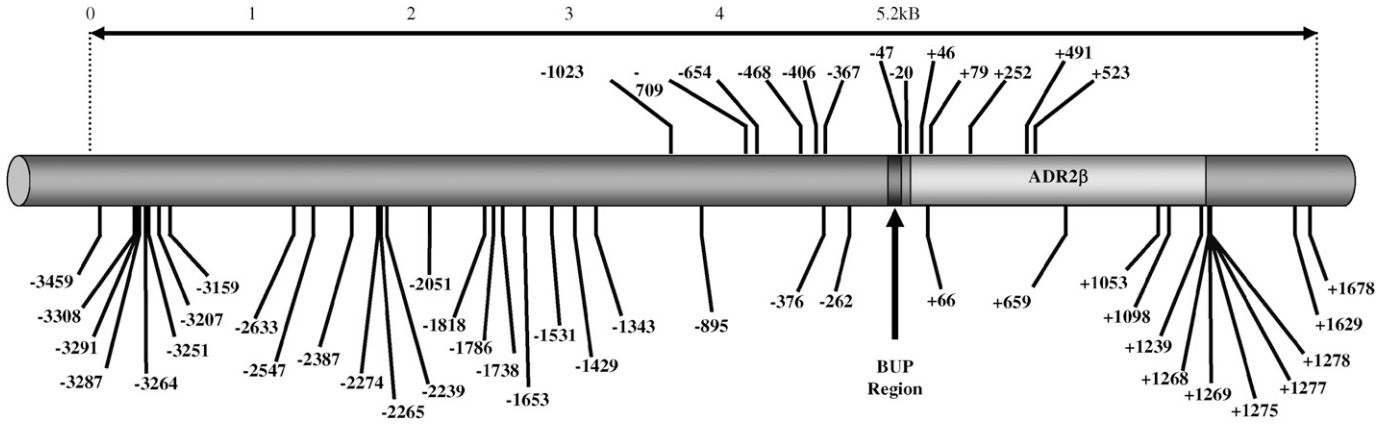

Figure 1. Position of polymorphisms in *ADR*β*2*.

from 9–15 Cs, based on fragment analysis (*see* online supplement). This poly-C region was interrupted by polymorphisms at +1268 and +1277 (positions based on reference sequences). Based on sequence analysis, the poly-C length variation occurs between polymorphisms +1268 and +1277. Statistical analysis of poly-C lengths revealed a predominant bimodal distribution in whites at 11 Cs (frequency = 0.40) and 13 Cs (frequency = 0.34), whereas African Americans have predominantly 13 Cs (frequency = 0.64; Table E2).

*ADR*β*2* Variants Exist as Multiple Haplotypes

LD (as D′ and r^2) was calculated separately for African Americans and whites using the polymorphisms used to define haplotype frequency (Table 2), with the exception of −709 (African American and white; frequency = 0) and +659 (whites; frequency = 0). Three additional polymorphisms (+1275, +1277, and +1278) were added to provide coverage in the 3′ UTR. D′ reflects whether a polymorphism is inherited in conjunction with a second polymorphism; however, it does not imply that the alleles at one polymorphism can predict the inheritance of an allele at another polymorphism. The correlation coefficient, r^2, is calculated to reflect the relationship between two polymorphisms, and whether individual alleles can predict the inheritance of each other. LD (*see* Figures E1–E4) was generally strong across *ADR*β*2* in both populations, except between rare polymorphisms in the 3′ region of *ADR*β*2*, and was lower in African Americans. For example, LD between +46 and 5′ polymorphisms (−3459, −3291, −3287, −2633, −2387, −2274, −1343, and −1023) was 0.95 or greater (D′), with an r^2 of 0.44 or greater, whereas, in whites, D′ was 0.21 or less, with an r^2 of 0.04 or less. Similar values were observed for +79 (in strong LD with +46) and 5′ polymorphisms.

A total of 31 polymorphisms genotyped from sequencing data (429 whites and 240 African Americans) were used to estimate haplotype frequency (Table 2). Polymorphisms used include those identified by Drysdale and colleagues (38) (except for −709 [frequency = 0 in this study]), polymorphisms identified in this study with frequencies of 0.03 or greater, and coding polymorphisms at +491 and +659. A total of 24 haplotypes with frequencies of 0.01 or greater were observed. When analysis was limited to the Drysdale polymorphisms, haplotypes 2, 4, 6, and 9 were observed in whites, whereas haplotypes 1, 2, 4, 6, 7, and 10 were observed in African Americans. Significant differences between Drysdale haplotype frequencies existed between whites and African Americans, especially haplotypes 1, 4, and 9, which encodes Arg^{16} at the +46 position. Drysdale haplotype 1 was only observed in African Americans, and Drysdale haplotypes 2 and 4 frequencies were higher in whites (frequency = 0.38 and 0.37, respectively) compared with African Americans (frequency = 0.12 and 0.21, respectively). Finally, Drysdale haplotype 6 was more than twice as frequent (frequency = 0.28) in African Americans than in whites (frequency = 0.12). Based on extended haplotype analyses, we were able to divide Drysdale haplotype 1 into three subhaplotypes, haplotype 2 into seven subhaplotypes, haplotype 4 into eight subhaplotypes, haplotype 6 into five subhaplotypes, and haplotype 10 into two subhaplotypes. Only one form of Drysdale haplotypes 7 and 9 were observed. All subhaplotypes were differentiated by polymorphisms in the 3′ portion of *ADR*β*2*, except for subhaplotypes 2-6, 4-8, and 6-2. Drysdale haplotypes 2 and 4 accounted for the majority of white haplotypes (frequency = 0.38 and 0.37, respectively), whereas haplotype frequencies in African American were more evenly distributed between the four Drysdale haplotypes 1, 2, 4, and 6 (frequency = 0.17, 0.12, 0.21, and 0.28, respectively).

Association Results

*ADR*β*2* polymorphisms −3459, −2387, −1818, −468, −47, +46, +79, +491, +523, +659, +1053, and +1269 were selected for genotyping in the adult asthma case and control population These polymorphisms, except for +491 and +659 (Table 3, highlighted in *boxes*) represented an optimal tagging subset based on LD and haplotype analysis. Coding polymorphisms +491 and +659 were included because of their potential effect on receptor function. Association studies including the +1269 poly-C polymorphism were performed by dividing +1269 poly-C lengths into two pooled groups—short lengths (S) (poly-Cs ⩽ 12 Cs) and long lengths (L; poly-Cs ⩾ 13 Cs)—based on the bimodal distribution of poly-C length in whites. The genotyping success rate ranged from a low of 95.5% (single-nucleotide polymorphism [SNP] −3459) to greater than 99% (SNP −47), and there were no significant differences in genotyping success rates between whites and African Americans. Adjustments were made for age and sex. All subjects with asthma were adults with less than 5 pack-years of smoking. Adjustments for current medications were not made, although subjects withheld medications prior to testing.

For African Americans, polymorphism +79 was found to be significantly associated with $(FEV_1/FVC)^2$ after adjusting for multiple testing ($p = 0.028$; $p = 0.007$ before adjustment) (genotypes N: CC = 167, CG = 80, GG = 11; $[FEV_1/FVC]^2 = 0.596$ [SD, ± 0.16], 0.586 [SD, ± 0.16], 0.543 [SD, ± 0.16], respectively; Table E3). Associations with % predicted FEV_1 (+79; $p = 0.034$), % predicted FVC (+79; $p = 0.05$), bronchodilator reversibility (+79; $p = 0.02$), and PC_{20} (+1,269 S vs. L; $p = 0.03$) were

TABLE 1. *ADRβ2* POLYMORPHISMS IN WAKE FOREST PLUS CAMP STUDY POPULATIONS

Polymorphism	dbSNP rs No.	Description of change	Major/Minor Allele	White Minor Allele Frequency	African-American Minor Allele Frequency
−3459	rs9325122		T/C	0.42	0.16
−3308			A/C	0.001	0.03
−3291	rs11957351		T/C	0.44	0.45
−3287	rs11948371		A/T	0.44	0.45
−3264			C/T	0.001	0.004
−3251	rs11960649		C/A	0.44	0.44
−3207			T/C	0.008	0
−3159			T/C	0.14	0.19
−2633	rs1432622		C/T	0.44	0.43
−2547			G/T	0.002	0
−2387	rs1432623		T/C	0.45	0.45
−2274	rs11168068		T/C	0.44	0.45
−2265			C/G	0	0.002
−2239	rs11287644	Poly-A	D/I	ND	ND
−2051		4C/5C	D/I	0	0.05
−1818	rs17778257		A/T	0.38	0.24
−1786			G/C	0.001	0.002
−1738			C/T	0.004	0.002
−1653			G/A	0.001	0.007
−1531	rs2400706		C/T	0.17	0.32
−1429	rs2895795		T/A	0.18	0.32
−1343	rs2400707		G/A	0.44	0.45
−1023	rs2053044		G/A	0.44	0.45
−895		Met^{31}Ile†	ATG>ATA	0.005	0
−709*		Val^{93}Val	GTC>GTA	NF	NF
−654	rs12654778	Glu^{112}Lys†	GAG>AAG	0.39	0.23
−468	rs11168070	His^{174}Asp†	CAC>GAC	0.44	0.19
−406		Pro^{194}Pro†	CCC>CCT	0.001	0.06
−376		25-base InDel‡	D/I	0	0.02
−367	rs11959427	Pro^{207}Pro†	CCC>CCT	0.44	0.16
−262		Glu^{242}Glu†	GAG>GAA	0.003	0.05
−47	rs1042711	Cys >Arg§	TGC>CGC	0.44	0.19
−20	rs1801704		T/C	0.44	0.19
46	rs1042713	Gly^{16}Arg	GGA>AGA	0.38	0.48
66		His^{22}His	CAC>CAT	0.004	0.007
79	rs1042714	Gln^{27}Glu	CAA>GAA	0.44	0.19
252	rs1042717	Leu^{84}Leu	CTG>CTA	0.17	0.32
491	rs1800888	Thr^{164}Ile	ACC>ATC	0.02	0.004
523	rs1042718	Arg^{175}Arg	CGC>CGA	0.15	0.32
659	rs3729943	Ser^{220}Cys	TCC>TGC	0	0.03
1053	rs1042719	Gly^{351}Gly	GGG>GGC	0.26	0.35
1098	(9)	Tyr^{366}Tyr	TAT>TAC	0.004	0
1239	rs1042720	Leu^{413}Leu	CTG>CTA	0.32	0.44
1268			C/G	0.002	0.06
1269		Poly-C	D/I	*See* Table E2	*See* Table E2
1275	rs1042721		C/G/A	0.29/0.001	0.54/0.06
1277			C/A	0.003	0.03
1278			C/A	0.005	0.05
1629	rs8192451		C/T	0.01	0
1678			G/A	0.003	0

Definition of abbreviations: dbSNP = NCBI Single Nucleotide Polymorphism database (dbSNP is online at: http://www.ncbi.nlm.nih.gov/SNP/); InDel = insertion/deletion; ND = not determined; NF = not found.

* Polymorphism listed but not found in this study.

† Predicted changes in peptide sequence in potential 5′ ORF.

‡ Twenty-five–base duplication (TCCAGGGAGCAGTTGGGCCCCGCCC).

§ leader cistron (LC) region.

also observed before adjusting for multiple testing (Table E3). Using the conservative approach of adjusting the p values by the number of tests performed, only $(FEV_1/FVC)^2$ remained significant. There was no evidence for associations with measures of pulmonary function in the white population.

Haplotype Association Results

Haplotype association tests were performed using the 12 polymorphisms genotyped in our adult case-control populations. Haplotypes were labeled using the Drysdale haplotype numbering convention (38), followed by a letter signifying the +1269 poly-C repeat length (A = 10 Cs, B = 11 Cs, etc.). Additional SNP identifiers indicate SNPs that further subdivide haplotypes. No significant haplotype associations were found in the white population.

Significant association of haplotypes was also observed with $(FEV_1/FVC)^2$ ($p = 0.006$) in African Americans. Individual haplotypes 2D and 4D were associated with $(FEV_1/FVC)^2$ ($p = 0.003$ and 0.019, respectively; Table 3). $(FEV_1/FVC)^2$ was also associated with the three SNP haplotype G/C/C (SNPs +79/+491/+523; frequency = 0.189; $p = 0.05$). Significant haplotype association was observed for % predicted FEV_1 ($p = 0.05$) in African Americans. Analysis of individual haplotypes revealed that haplotypes containing a 13 Cs repeat at +1269 were associated with % predicted FEV_1 (haplotypes 2D and 4D; $p = 0.002$

TABLE 2. HAPLOTYPE FREQUENCY IN WHITES AND AFRICAN AMERICANS

Drysdale Haplotype		Polymorphism																																			CCFEQ	AAFEQ
		−3459*	−3308	−3291	−3287	−3251	−3159	−2633	−2387*	−2274	−1818*	−1531	−1429	−1343	−1023	−709	−654	−468*	−406	−367	−262	−47*	−20	p46*	p79*	p252	p491*	p523*	p659*	p1053*	p1239	p1268	+1269*		
1	1-1	T	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	C	C	T	G	T	T	A	C	G	C	C	C	G	A	C	13	0.00	0.14
	1-2	T	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	C	C	T	G	T	T	A	C	G	C	C	C	G	A	C	**14**	0.00	0.02
	1-3	T	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	C	C	T	G	T	T	A	C	G	C	C	C	G	**G**	**G**	**12**	0.00	0.01
																																				0.00	0.17
2	2-1	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	G	C	C	G	G	G	C	C	C	G	G	C	11	0.30	0.04
	2-2	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	G	C	C	G	G	G	C	C	C	G	G	C	**10**	0.03	0.00
	2-2	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	G	C	C	G	G	G	C	C	C	G	G	C	**12**	0.02	0.01
	2-4	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	G	C	C	G	G	G	C	C	C	G	**A**	C	**13**	0.02	0.00
	2-5	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	G	C	C	G	G	G	C	C	C	**C**	**A**	C	**13**	0.01	0.01
	2-6	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	**A**	C	C	G	G	G	C	C	C	G	G	C	**13**	0.00	0.04
	2-7	C	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	G	C	C	G	C	C	G	G	G	C	C	C	G	G	C	**13**	0.00	0.02
																																				0.38	0.12
4	4-1	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	G	G	C	12	0.13	0.04
	4-2	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	G	G	C	**13**	0.07	0.07
	4-3	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	**C**	**A**	C	**13**	0.07	0.01
	4-4	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	G	G	C	**11**	0.05	0.06
	4-5	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	G	**A**	C	**13**	0.02	0.01
	4-6	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	G	**A**	C	12	0.02	0.00
	4-7	T	A	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	**C**	**A**	C	**14**	0.01	0.00
	4-8	T	**C**	T	A	C	T	C	T	T	T	C	T	G	G	C	A	C	C	T	G	T	T	A	C	G	C	C	C	G	G	C	**11**	0.00	0.02
																																				0.37	0.21
6	6-1	T	A	T	A	C	C	C	T	T	A	T	A	G	G	C	G	C	C	T	G	T	T	G	C	A	C	A	C	C	A	C	13	0.09	0.18
	6-2	T	A	T	A	C	**T**	C	T	T	A	T	A	G	G	C	G	C	C	T	G	T	T	G	C	A	C	A	C	C	A	C	13	0.02	0.10
	6-3	T	A	T	A	C	C	C	T	T	A	T	A	G	G	C	G	C	C	T	G	T	T	G	C	A	C	A	C	**G**	**G**	C	**12**	0.01	0.00
																																				0.12	0.28
7	7-1	T	A	T	A	C	T	C	T	T	A	T	A	G	G	C	G	C	C	T	G	T	T	G	C	A	T	A	C	C	A	C	13	0.02	0.00
																																				0.02	0.00
9	9-1	T	A	C	T	A	T	T	C	C	A	C	T	A	A	C	G	C	T	T	G	T	T	A	C	G	C	C	C	G	G	G	12	0.00	0.03
																																				0.00	0.03
10	10-1	T	A	T	A	C	C	C	T	T	A	T	A	G	G	C	G	C	C	T	G	T	T	G	C	A	C	C	C	C	**A**	C	**13**	0.02	0.00
																																				0.02	0.00

Definition of abbreviations: AAFEQ = haplotype frequency in African Americans; CCFEQ = haplotype frequency in whites.

Frequency ⩾ 0.01. Letters within the *boxes* indicate polymorphisms studied by Drysdale and coworkers (38) and Taylor and coworkers (56). Letters in *bold* indicate SNPs that define subhaplotypes.

* Tagging SNPs used in association study.

and 0.01, respectively; Table E4). A three-SNP sliding window analysis identified a single three-SNP haplotype (C/G/13; SNPs +659/+1053/+1269; frequency = 0.326) that was associated with percent- predicted FEV_1 ($p = 0.014$). Global association of haplotypes with % predicted FVC was not significant in African Americans. However, individual haplotypes 4C and 4D (Table E5) were associated with % predicted FVC ($p = 0.02$ and 0.025, respectively). Haplotypes 4C and 4D were differentiated by the length of the poly-C region at +1269. This analysis suggests that the length of the poly-C region may influence lung function in African Americans. A comparison of Drysdale haplotype pairs (38) with acute reversibility to albuterol found no significant correlations between haplotype pairs and percent reversibility (data not shown).

DISCUSSION

In previous studies, DNA sequence and haplotype analysis of *ADR*β*2* has been limited to a region approximately 1,430 bp 5′ of the ATG start codon to approximately 1,200 bp 3′ of the ATG start codon, leaving a significant portion of 5′ regulatory sequence and 3′ UTR uncharacterized (9, 38, 52–54). Many of these studies were inconsistent in their findings due to the smaller region of the gene that was sequenced and the limited size of the population samples. Recent clinical studies suggest that there may be a subset of patients with asthma, especially African Americans, who have adverse responses to β-agonist therapy (39, 55). Our primary purpose in this study was to comprehensively resequence a 5.3-kb region of *ADR*β2 in 669 African American and white subjects. We identified 49 polymorphisms, 20 of which have not been previously reported, and found variations in regulatory regions, including the promoter region and the 3′ UTR, that contribute to haplotypic structure and to genetic association with asthma-related phenotypes.

Whereas LD, as measured by D′, is strong across the entire gene in both African American and white populations, measurements of r^2 between 5′ polymorphisms and +46 and +79 polymorphisms were not concordant between the two ethnic groups. For example, whites do not possess haplotype 1, which contains Arg^{16} (Table 3), whereas the frequency of haplotype 1 approaches 20% in African Americans. Haplotype 1 appears to have originated from a combination of haplotypes 2 and4, but only involving the polymorphisms −3291 to −654 (38). The consequence of this recombination is that African Americans possessing an Arg^{16}/Gln^{27} haplotype are equally divided in frequency between haplotypes 1 and 4 (Table 3), whereas whites possessing an Arg^{16}/Gln^{27} haplotype always have haplotype 4.

TABLE 3. HAPLOTYPE ASSOCIATION WITH $(FEV_1/FVC)^2$ IN AFRICAN AMERICANS

	Polymorphism														
Haplotype	−3459	−2387	−1818	−468	−47	+46	+79	+491	+523	+659	+1053	+1269	Frequency	Hap-Score	p Value
1C	T	C	A	C	T	A	C	C	C	C	G	12	0.035	−0.362	0.717
1D	T	C	A	C	T	A	C	C	C	C	G	13	0.171	0.337	0.736
1E	T	C	A	C	T	A	C	C	C	C	G	14	0.013	0.780	0.436
2A	C	C	A	G	C	G	G	C	C	C	G	10	0.012	−2.146	0.034
2B	C	C	A	G	C	G	G	C	C	C	G	11	0.064	0.996	0.319
2C	C	C	A	G	C	G	G	C	C	C	G	12	0.017	−0.574	0.566
2D	C	C	A	G	C	G	G	C	C	C	G	13	**0.063**	**−2.939**	**0.003**
2D −3459	T	C	A	G	C	G	G	C	C	C	G	13	0.018	−2.396	0.017
4B	T	T	T	C	T	A	C	C	C	C	G	11	0.089	1.382	0.167
4C	T	T	T	C	T	A	C	C	C	C	G	12	0.055	0.483	0.629
4D	T	T	T	C	T	A	C	C	C	C	G	13	**0.080**	**−2.351**	**0.019**
4D +1053	T	T	T	C	T	A	C	C	C	C	C	13	0.019	−0.417	0.677
6D	T	T	A	C	T	G	C	C	A	C	C	13	0.233	1.133	0.257
6D +659	T	T	A	C	T	G	C	C	A	G	C	13	0.035	1.302	0.193
6E	T	T	A	C	T	G	C	C	A	C	C	14	0.015	0.072	0.942
							G	C	C				**0.189**	**−1.962**	**0.050**
							C	C	C				0.510	0.259	0.796
							C	C	A				0.293	1.619	0.105

Bold text indicates significantly associated haplotypes. Global p value = 0.0006.

Therefore, the 5′ region of the gene will be different approximately 50% of the time when comparing African American individuals with the Arg^{16}/Gln^{27} haplotype. Taking into account additional 3′ UTR differences that occur due to variations in the poly-C region at +1269, regulation of gene expression and translation in African Americans could be more complex than in whites. These findings are potentially important considering recent studies showing increased mortality especially in African Americans during long-term β-agonist therapy (55).

In addition to haplotype differences that may affect gene regulation, we also identified sequence variants unique to the African American subjects. Polymorphism +659 ($Ser^{220}Cys$) was found in 3% of African Americans, with no homozygotes identified. This mutation occurs at the boundary of the cell membrane and the third intracytoplasmic loop, directly adjacent to a peptide region assigned to G_s coupling regulation. We could not find prior evidence that this mutation has been characterized functionally; thus, the possible biological effects of this mutation are unknown. We also identified a large InDel at −376 in African Americans (frequency = 0.02). This InDel, a duplication of 25 bases, occurs adjacent to a G-rich region at position −365 to −325, but does not alter the potential regulatory region. We can only speculate that this InDel could have effects on gene expression. There were no individuals homozygous for −376. Interestingly, polymorphisms −376 and +659 were never found in an individual, although nearly 5% of the African Americans carried one of these polymorphisms. In these individuals, there is the potential of alterations in *ADRβ2* expression or receptor function.

Single SNP associations observed with lung function phenotypes varied between ethnic groups. We did find an association between the +79 SNP and $(FEV_1/FVC)^2$, with the G/G subjects having lower values in African Americans. Interesting, +79 C/G heterozygous individuals did not show lower pulmonary function, suggesting a protective effect of the Gln^{27} allele, which has been suggested in previous *in vitro* studies (10). These results are consistent with the family-based results reported by Silverman and coworkers (37).

Drysdale and coworkers (38) were able to identify 12 haplotypes using a subset of 13 polymorphisms. Using the same subset of polymorphisms in our larger set of subjects, we were able to identify only seven of these haplotypes. There are multiple explanations for these discordant results, including the smaller number of subjects sequenced from each ethnic group in the Drysdale study, the absence of polymorphism −709 in our population samples, the estimation of haplotypes by different algorithms, and inclusion of Asians and Hispanics in the Drysdale study. Because we resequenced a larger set of subjects, some of the missing haplotypes may also be accounted for by very rare haplotypes (frequency < 0.01) that we excluded in our study, but were found in one of the Drysdale subjects. For instance, based on haplotype analysis (Table 3), 81% of African Americans (194 of 240) had the most common Drysdale haplotypes. An important component of our haplotype analysis is the subdivision of the "functionally" important Drysdale haplotypes 1, 2, and 4. Drysdale haplotypes 1and 4, both of which contain the risk Arg^{16} allele, were divided into three and eight subhaplotypes, respectively; however, only haplotype 1 was present in African Americans. With the addition of Drysdale haplotype 9 (occurring only in African Americans), there are 10 extended haplotypes (frequency ≥ 0.01) in African Americans that contain the risk Arg^{16} allele. Drysdale haplotype 2, which was the most common in whites, was divided into seven subhaplotypes. With the exceptions of extended haplotypes 2-6 and 4-8 (Table 3), all Drysdale haplotype subdivision was caused by variations at polymorphisms +1053, +1239, +1268, and the +1269 poly-C region in the 3′ UTR of *ADRβ2*, none of which have been included in prior haplotype analyses of *ADRβ2*. Belfer and colleagues (51) have recently reported a haplotype analysis of *ADRβ2* in 96 whites and 96 African Americans using 11 polymorphisms. However, because of the small number of polymorphisms used in that study and the extragenic location of some polymorphisms, a direct comparison of these results with ours and those of the Drysdale study (38) is not possible.

We did not find an association between haplotype pairs and acute bronchodilator response that was reported by Drysdale. A recent report by Taylor and coworkers (56) also found no significant correlations between Drysdale haplotype pairs and bronchodilator response to albuterol in 176 subjects with asthma in New Zealand (38). Although we did not observe significant association of haplotypes with percent-predicted FEV_1 and percent predicted FVC in African Americans, the analysis revealed an interesting trend in the association of poly-C lengths in

polymorphism +1269 with FEV_1 and percent-predicted FVC. For both measures, haplotypes 4C and 4D were significantly associated with lung function, but with opposite Hap-scores. The only difference between haplotypes 4C and 4D are the lengths of the +1,269 poly-C region. There is a trend for haplotype 2, with increasing the poly-C length from 11 to 13, to be associated with lower percent-predicted FEV_1 and $(FEV_1/FVC)^2$. This poly-C region at +1269 lies only 20 bp 5′ of an AU-rich element (ARE), which has been shown to affect mRNA stability and mRNA translation rates of several other genes (57–60). Tholanikunnel and coworkers have shown that DNA sequence changes in a similarly positioned ARE in the guinea pig *ADR*β*2* 3′ UTR modulates the translation rates of β_2-receptor protein, reducing receptor density in the cell membrane (61). It is possible that this poly-C region could have an independent effect or a cooperative effect with the flanking ARE region in modulating mRNA stability and protein translation rates. This could explain the discrepancy in the Drysdale study that was observed for bronchodilator reversibility between haplotype pairs, as the poly-C region variation was not evaluated in the Drysdale haplotypes (38). The interruptions at positions 3 (+1268) and 10 (+1275) may also be important determinants modulating the poly-C region effects on gene expression. Although polymorphism +1268 is relatively rare, polymorphism +1275, has three alleles, is very common (frequency range, 0.29–0.54), mostly occurs in poly-C repeats of 13, 14, and 15 Cs, and further subdivides haplotypes containing long poly-C repeats (data not shown). If poly-C region variations do alter gene expression and/or translation, the use of *ADR*β*2* polymorphisms to predict β-agonist response will be complicated by the large number of haplotypes that must be considered.

The primary focus of this study was to accurately determine polymorphism content and haplotype structure in whites and African Americans; thus, the association results are considered secondary. However, we must note that there are several factors that affect the association results in this study. First, the degree of population stratification in these case and control populations has not been measured; thus, we do not know if population stratification affects these association results. However, it is not clear what effects population stratification may have on quantitative analyses that were performed in this study. This is particularly true in the African Americans, where population stratification can be more pronounced. Second, although our African American subjects constitute the largest case-control population evaluated for effects of *ADR*β*2* variation and lung function, this population may still be underpowered for this type of association study. Finally, because numerous phenotypes were tested, multiple comparison adjustments reduced the significance of genetic primary associations. However, the measures of lung function FEV_1, FVC, and $(FEV_1/FVC)^2$ are correlated, so our adjustments were conservative.

In this study, we have characterized *ADR*β*2* in two ethnic populations demonstrating several new sequence features of the gene, and have identified a minimum of 27 haplotypes. Thus far, simple genotyping of the +46 ($Gly^{16}Arg$) and +79 ($Gln^{27}Glu$) *ADR*β2 polymorphisms has been used to characterize specific β-agonist responses in a number of pharmacogenetic studies (30, 32, 62). The results of this study, however, suggest that additional genetic variants in *ADR*β2 may be important in asthma association and pharmacogenetic studies. There is also the possibility that genetic variants in *ADR*β2 will be more useful in more complex genomic studies investigating gene–gene interactions with other genes in the *ADR*β2 pathway. Although many of the new polymorphisms identified in this study are rare or are not found in all ethnic populations, the interindividual and interracial pharmacogenetic effects that these rare polymorphisms may have on *ADR*β2 expression or receptor function may be significant. Thus, the results of this study support the need for complete genetic analysis of *ADR*β2 in current and future pharmacogenetic studies that evaluate the relationship of *ADR*β2 variants and responses to the acute or chronic administration of β-agonists. This study also demonstrates the genetic complexity that can be missed by genotyping only a few polymorphisms in a biologically or pharmacologically important gene. Although it may not be practical to sequence every portion of a gene, this study demonstrates the importance of sequencing a minimum, predefined region of the gene promoter region, all of the coding region, and the potential regulatory region in the 3′ UTR. This is particularly important considering recent reports that severe adverse events and even increased mortality, although infrequent, have been observed, especially in African Americans receiving long-term β-agonist therapy (39, 55). Thus, it is possible that variation in *ADR*β2 may represent an underlying genetic mechanism that contributes to variation in therapeutic responses and, possibly, adverse responses associated with chronic β-agonist therapy.

***Conflict of Interest Statement*:** G.A.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; K.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; D.A.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.J.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; W.C.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; B.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.B.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.P.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.T.W. received a grant for $900,065 (Asthma Policy Modeling Study) from AstraZeneca from 1997–2003, has been a coinvestigator on a grant from Boehringer Ingelheim to study a chronic obstructive pulmonary disease natural history model, which began in 2003, and has received no funds for his involvement in this project. He has been an advisor and chair on the advisory board to the TENOR Study for Genentech, and has received $10,000 for 2005–2003. He received a grant from Glaxo-Wellcome for $500,000 for genomic equipment from 2000–2003. He was a consultant for Roche Pharmaceuticals in 2000, and received no financial renumeration for this consultancy. He has also served as a consultant to: Pfizer (2000–2003), Schering Plough (1999–2000), Variagenics (2002), Genome Therapeutics (2003), and Merck Frost (2002); E.R.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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