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ARTICLE

Haplotype structure of the beta adrenergic receptor genes in US Caucasians and African Americans

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The beta-adrenergic receptors (β-AR) are G protein-coupled receptors activated by epinephrine and norepinephrine and are involved in a variety of their physiological functions. Previously, three β-AR genes (*ADRB1*, *ADRB2* and *ADRB3*) were resequenced, identifying polymorphisms that were used in genetic association studies of cardiovascular and metabolic disorders. These studies have produced intriguing but inconsistent results, potentially because the known functional variants: *ADRB1* Arg389Gly and Gly49Ser, *ADRB2* Arg16Gly and Gln27Glu, and *ADRB3* Arg64Trp provided an incomplete picture of the total functional diversity at these genes. Therefore, we created marker panels for each β-AR gene that included the known functional markers and also other markers evenly spaced and with sufficient density to identify haplotype block structure and to maximize haplotype diversity. A total of 27 markers were genotyped in 96 US Caucasians and 96 African Americans. In both populations and for each gene, a single block with little evidence of historical recombination was observed. For each gene, haplotype captured most of the information content of each functional locus, even if that locus was not genotyped, and presumably haplotype would capture the signal from unknown functional loci whose alleles are of moderate abundance. This study demonstrates the utility of using β-AR gene haplotype maps and marker panels as tools for linkage studies on β-AR function.
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Introduction

The human beta-adrenergic receptors (β-AR) are G_s-protein-coupled receptors that bind catecholamine neurotransmitters and signal transduce by raising intracellular levels of cyclic AMP.[1] β-AR are implicated in a variety of catecholamine-mediated physiological functions and in the pathophysiology of obesity,[2] asthma[3] and cardiovascular disorders.[4] β-AR have been classified into β1, β2 and β3 subgroups. β1 receptors are expressed in the heart, kidney, blood vessels and regulate heart rate and vascular tone. β2 receptors are widely distributed in the respiratory tract, and relax smooth muscle in small airways. β3 receptors are found mainly in adipose tissue, where they stimulate lipolysis and thermogenesis.[5] Genes encoding the three β-AR subtypes (*ADRB1*, hCG39839; *ADRB2,* hCG36934 and *ADRB3,* hCG21141) are located on chromosomes 10, 5 and 8, respectively. *ADRB1* and *ADRB2* are

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nonintronic, and are 3.2 and 3.4 kb, respectively. *ADRB3* has one intron and is 3.7 kb in length.

Functional loci have been identified at each of the β-AR genes. Two abundant *ADRB1* missense variants Ser49Gly and Gly389Arg[6] alter *in vitro* receptor coupling[7] but have no clear *in vivo* significance. Some positive results were reported using these markers in linkage studies of cardiomyopathy,[8] heart and renal failure,[7,9] and hypertension.[10] However, no relationship was detected to the response of healthy subjects to drugs acting through the β1-AR[11] nor in the hemodynamic response of hypertensive subjects to chronic β1-AR blockade.[12] Two obesity studies with the Gly389Arg marker yielded conflicting results.[13,14]

Within the coding region of *ADRB2*, nine SNPs were identified,[15] five of which are synonymous. Missense substitutions were Arg16Gly, Gln27Glu, Val34Met, and Thr164Ile.[16,17] Among them, two common alleles have been shown to be functional *in vitro*: Gly16 leads to enhanced agonist-mediated downregulation, and Glu27 reduces such regulation.[18] These polymorphisms have been associated with a variety of β-AR-related phenotypes, but association results have been inconsistent across different studies. Arg16Gly was associated with obesity,[19,20] diabetes[21] and cystic fibrosis,[22] but not with plasma norepinephrine concentration[23] or agonist-induced beta 2AR desensitization.[24] Evidence regarding the Arg16Gly polymorphism's relationship with asthma is conflicting.[25,26] Gln27Glu was associated with hypertriglyceridemia[27] and obesity in Spanish men[28] but not in the Tongan population.[29]

ADRB3 Trp64Arg is located in the first intracellular loop of the receptor. Arg64 has higher allele frequencies in Pima Indians [0.31] as compared to Mexican Americans [0.13], African Americans [0.12], and Caucasians [0.08][30] supporting the idea that this variant could impair activation of thermogenesis in adipose cells, contributing to the high frequency of obesity and adult onset diabetes in the Pima Indians.[31] However, the linkage studies are contradictory.[32]

Taken together, this evidence illustrates that a consistent picture of β-AR genotype–phenotype relationships has yet to emerge. Other functional loci may be present, including polymorphisms which are known but which have not yet been recognized to be functional. A haplotype approach combining known functional polymorphisms with a series of loci chosen for haplotype informativeness could comprehensively capture the potential information content on β-AR functional variants of moderate abundance.[33] In this study, we report a haplotype map for each of the β-AR genes for two populations, American Caucasians and African Americans, by genotyping a panel of SNP markers and the known functional polymorphisms in these populations. For each gene, we also describe marker panels that maximize haplotype information content.

Materials and methods

Participants

A total of 192 unrelated subjects were genotyped, including 96 individuals from each of two populations: US Caucasians and African Americans. Informed consent was obtained according to human research protocols approved by the human research committees of the recruiting institutes, including the National Institute on Alcohol Abuse and Alcoholism, National Institute of Mental Health and Rutgers University. All participants had been psychiatrically interviewed and none had been diagnosed with a psychiatric disorder.

SNP markers

The physical position and frequency of minor alleles ($>$0.05) from a commercial database (Celera Discovery System, CDS, September, 2003) were used to select SNPs (including known nonsynonymous substitutions). 5′ nuclease assays (*vide infra)* were designed for seven *ADRB1*, 11 *ADRB2* and nine *ADRB3* SNPs and optimized. These markers were nearly equally spaced and covered the entire genes plus 2.5–6 kb upstream and 2.5–6 kb downstream from each gene.

Genomic DNA

Genomic DNA was extracted from lymphoblastoid cell lines, diluted to a concentration of 10 ng/μl. Aliquots of 1 μl were dried in 384-well plates.

Polymerase chain reaction (PCR) amplification

Genotyping was performed by the 5′ nuclease method[34] using fluorogenic allele-specific probes. Oligonucleotide primer and probe sets were designed based on gene sequence from the CDS, September 2003. Primers and detection probes for each locus in each gene are listed in Table 1a–c.

Reactions were in a 5 μl volume containing 2.375 μl TE, 2.5 μl Master Mix (ABI, Foster City, CA, USA) with AmpliTaq Gold® DNA Polymerase, dNTPs, Gold Buffer and $MgCl_2$, 10 ng genomic DNA, 900 nM of each forward and reverse primer and 100 nM of each reporter and quencher probe. DNA was incubated at 50°C for 2 min and at 95°C for 10 min, and amplified on an ABI 9700 device for 40 cycles at 95°C for 30 s and 60°C for 75 s. Allele-specific signals were distinguished by measuring end point 6-FAM or VIC fluorescence intensities at 508 and 560 nm, respectively, and genotypes were generated using Sequence Detection V.1.7 (ABI).

Genotyping error rate was directly determined by regenotyping 25% of the samples, randomly chosen, for each locus. The overall error rate was $<$0.005. Genotype completion rate was 0.99.

Table 1 Primer and probe sequences for 5′ nuclease genotyping

SNP	*Primers and probes*	*Sequences*
(a) Of seven *ADRB1* markers		
1	Assay on Demand # 2648572 (ABI, Ca)	
2	Forward primer	TCTCAAAAAAATAAAAAGAAAGAAGCCAGGAG
	Reverse primer	GGAGACTACACCCCAAGTTATCCAA
	Allele 1 probe (FAM)	CAGGCTTGCAGTGAC
	Allele 2 probe (VIC)	TCTCAGGCTTTCAGTGAC
3	Forward primer	CCGCCCGCCTCGTT
	Reverse primer	CGCTGTCCACTGCTGAGA
	Allele 1 probe (FAM)	CAGCGAAGGCCCCGA
	Allele 2 probe (VIC)	CCAGCGAAAGCCCCGA
4	Forward primer	CCGCAGCCCCGACTTC
	Reverse primer	GCCGGTCTCCGTGGGT
	Allele 1 probe (FAM)	CTTCCAGGGACTGC
	Allele 2 probe (VIC)	TTCCAGCGACTGCT
5	Forward primer	ACATTTTCACAGCCGGATTCA
	Reverse primer	TCTTCAGTCAAGTGCAGCAGATG
	Allele 1 probe (FAM)	AGGTGTTCTAGATTACTT
	Allele 2 probe (VIC)	AGGTGTTCTAGACTACTT
6	Forward primer	CTTGTTAAGCACATTTTCCTAGCTTATGTT
	Reverse primer	GTGCAGAATTTCTGTCTGGCAATTT
	Allele 1 probe (FAM)	CAGAGGAACGGGCACA
	Allele 2 probe (VIC)	CCAGAGGAACAGGCACA
7	Forward primer	GTTGTTCCACCTTCAACTATTTGCA
	Reverse primer	GGCCATCTGCTTGAAAGAGTTTT
	Allele 1 probe (FAM)	ATTTGTGGGAATTGT
	Allele 2 probe (VIC)	TGATTTGTGAGAATTGT
(b) Of 11 *ADRB2* markers		
1	Assay on Demand # 2084740 (ABI, Ca)	
2	Forward primer	CAAGTTGTTGTGTAGGATATTGGCAATT
	Reverse primer	GTGCTTTGAGGGCCACTGA
	Allele 1 probe (FAM)	CGAATCAGAAATTTA
	Allele 2 probe (VIC)	CCGAATCAAAAATTTA
3	Forward primer	TCCAGTTCAAATGAAGCATTAACTCTCT
	Reverse primer	CCAGCAGAGGAGTTCGAGTAG
	Allele 1 probe (FAM)	ATGTGAACAGTATGCAGTG
	Allele 2 probe (VIC)	ATGTGAACAGTAAGCAGTG
4	Forward primer	TGAGGTGAGTGTATTTTGAAAACTATGTGA
	Reverse primer	TGCAAGACAGATGCCTTAGAAAACA
	Allele 1 probe (FAM)	ACAAATATGAATTAAGGATCTA
	Allele 2 probe (VIC)	CACAAATATGAATTAAAGATCTA
5	Forward primer	AACCACTAAGTAATTTATGTAAACTTCGCT
	Reverse primer	TAAGAAATATGAAAATGCTTTTGCTCAT
	Allele 1 probe (FAM)	CACACAAGTGTAGTTTG
	Allele 2 probe (VIC)	TCACACAAGTATAGTTTG
6	Forward primer	GTAAGTCACAGACGCCAGATGGT
	Reverse primer	CCTTTCATCTGCTGGATAGTTTGTT
	Allele 1 probe (FAM)	ATGGCACAACCCG
	Allele 2 probe (VIC)	ACATGGCGCAACC
7	Forward primer	CGGCAGCGCCTTCTTG
	Reverse primer	TGCGTGACGTCGTGGTC
	Allele 1 probe (FAM)	ACCCAATGGAAGCC
	Allele 2 probe (VIC)	CACCCAATAGAAGCC
8	Forward primer	CCTTCTTGCTGGCACCCAAT
	Reverse primer	TGCCCACCACCCACAC
	Allele 1 probe (FAM)	CGTCCCTTTGCTGCGT
	Allele 2 probe (VIC)	CTCGTCCCTTTCCTGCGT
9	Assay on Demand # 2084766 (ABI, Ca)	
10	Forward primer	CCTGCGCAGGTCTTCTTTG
	Reverse primer	GTGTTGCCGTTGCTGGAGTA
	Allele 1 probe (FAM)	CTATGGCAATGGC
	Allele 2 probe (VIC)	AGGCCTATGGGAATG
11	Forward primer	TGAGAAATTCTGGTGTTTTGTGAATAA
	Reverse primer	GGTGGTGGGTGGGAGGTT

Table 1 Continued

SNP	Primers and probes	Sequences
	Allele 1 probe (FAM)	TGAAAAGAGGCCCC
	Allele 2 probe (VIC)	TGAAAAGAGCCCCC
(c) Of nine *ADRB3* markers		
1	Assay on Demand # 3273558 (ABI, Ca)	
2	Forward primer	AGAGCCTGGAGAACACTAAGGT
	Reverse primer	GTGAGTGCTTAGGGCAAAGAGA
	Allele 1 probe (FAM)	CCATTCCTTCTGCCACC
	Allele 2 probe (VIC)	TTCCATTCCTTTTGCCACC
3	Assay on Demand # 3273557 (ABI, Ca)	
4	Assay on Demand # 3273556 (ABI, Ca)	
5	Forward primer	GCTTCCCGACCCTGAGC
	Reverse primer	GCAGCCCAGGCTTTGC
	Allele 1 probe (FAM)	TCTGCCCCGGTTAC
	Allele 2 probe (VIC)	CCTCTGACCCGGTTAC
6	Assay on Demand # 2215549 (ABI, Ca)	
7	Forward primer	CAACTCCCTCGGTGCCA
	Reverse primer	CCCCTTTAAGCGTCGCTACTC
	Allele 1 probe (FAM)	CTCCCCCGAGAGCG
	Allele 2 probe (VIC)	TCCCCCAAGAGCG
8	Forward primer	CTGGGAGTTAGGAAGGTTGCA
	Reverse primer	GGAGACGAGGCTGGTTCTTT
	Allele 1 probe (FAM)	CTAAAATTATCCCCAAGGAA
	Allele 2 probe (VIC)	CTAAAATTATCCCGAAGGAA
9	Forward primer	CTTTGTTCTGTGCCCTTGGAA
	Reverse primer	GGCCTGAACTCAGTGCATT
	Allele 1 probe (FAM)	ACAGACGCTGCCTG
	Allele 2 probe (VIC)	CACAGATGCTGCCTG

Haplotype analysis

Haplotype frequencies were estimated using a Bayesian approach implemented with PHASE.[35] These frequencies closely agreed with results from a maximum likelihood method implemented via an expectation-maximization (EM) algorithm.[36] Haploview version 2.0.2 (Whitehead Institute for Biomedical Research, USA) was used to produce LD matrices.

Results and discussion

Of a total of 27 markers in three β-AR genes, 23 were polymorphic both in US Caucasians and African Americans. *ADRB3* marker #2 (rs4999) was monomorphic in Caucasians, and *ADRB3* markers 7–9 (rs4993, rs802162 and rs13258937) were monomorphic in both populations. Dramatic interpopulation differences in allele frequencies were observed for many of the markers. Allele frequencies of all markers and their locations in the genes are shown in Table 2a–c. For *ADRB1,* two functional nonsynonymous polymorphisms (Ser49Gly and Ala389Gly) are located in the exon, one marker is located in the gene 3′ UTR region, and the rest of the markers are in the intergenic region upstream and downstream of *ADRB1* (Figure 1a). For *ADRB2,* two functional nonsynonymous polymorphisms (Arg16Gly and Gln27Glu) and two synonymous polymorphisms are located in the exon, one marker is located in the gene 5′ UTR region, and the rest of the markers are in the intergenic region upstream and downstream of *ADRB2* (Figure 1b). For *ADRB3*, one functional nonsynonymous polymorphism (Arg64Trp) is located in exon 1, one marker is located in the 5′ UTR region (exon 1), two markers are located in the gene 3′ UTR region (exon 2), and the rest of the markers are in the intronic sequence and intergenic region upstream and downstream of *ADRB3* (Figure 1c).

Within the *ADRB1*, *ADRB2* and *ADRB3* regions, a single conserved haplotype block spanned each gene in both Caucasians and African Americans (Figure 2a–c) and the block boundaries extend beyond the region we have evaluated. In African Americans, the *ADRB2* block may be smaller; the first and last SNPs were in lower linkage disequilibrium (LD) [$D' < 0.8$] with all other markers. Definition of haplotype blocks and block boundaries is inexact. Isolated nucleotide substitutions can occur within nonrecombined blocks. On the other hand, some disruptions of LD occurring within blocks are attributable to low allele frequencies that lead to increased variance in estimation of LD. We discounted low D' values which might have originated from this cause. In the *ADRB1*,

Table 2 Locations and allelic frequencies in 96 individuals from each of two populations

#	SNP ID (CDS)	SNP ID (NCBI)	Variation	Position (CDS)	Location	Allelic frequency (for allele 2) US Caucasians	African Americans
(a) Of seven *ADRB1* markers							
1[a,b]	2648572	rs2773469	G>A	109526266	5′ Intergenic	0.316	0.4
2[a,b]	11743658	rs2053095	G>T	109528966	5′ Intergenic	0.926	0.681
3	8898508	rs1801252	Gly49Ser (G>A)	109531407	Exon	0.926	0.744
4[a,b]	27859421	rs1801253	Gly389Ala (G>C)	109532428	Exon	0.662	0.571
5[b]	2648569	rs3813719	T>C	109534254	3′ UTR/exon	0.907	0.962
6	16149641	rs2183378	G>A	109536263	3′ Intergenic	0.081	0.039
7[b]	2648567	rs7920400	G>A	109538512	3′ Intergenic	0.895	0.8
(b) Of 11 *ADRB2* markers							
1	2084740	rs879096	C>A	144279479	5′ Intergenic	0.777	0.41
2[a,b]	11830550	No rs	G>A	144281317	5′ Intergenic	0.664	0.772
3	2084751	rs11958940	T>A	144283535	5′ Intergenic	0.432	0.412
4	8950504	rs1432622	G>A	144285812	5′ Intergenic	0.42	0.41
5	2084757	rs1432623	G>A	144286058	5′ Intergenic	0.573	0.571
6	2084759	rs2400707	A>G Gly16Arg	144287102	5′ UTR/Exon	0.563	0.577
7	2084764	rs1042713	(G>A) Gln27Glu	144288490	Exon	0.341	0.448
8[a,b]	2084765	rs1042714	(G>C) Leu84Leu	144288523	Exon	0.386	0.186
9[a,b]	2084766	rs1042717	(A>G) Gly351Gly	144288696	Exon	0.239	0.37
10[a,b]	8950496	rs1042719	(C>G)	144289497	Exon	0.696	0.618
11[a]	2843228	rs7702861	G>C	144293909	3′ Intergenic	0.836	0.605
(c) Of nine *ADRB3* markers							
1[a,b]	3273558	rs9694197	G>A	36771802	3′ Intergenic	0.918	0.827
2[b]	12106155	rs4999	C>T	36773088	3′ UTR/Exon 2	0	0.067
3[b]	3273557	rs4998	G>C	36773195	3′ UTR/Exon 2	0.907	0.69
4	3273556	rs2071493	T>C	36773562	Intron 1	0.919	0.871
5	12106153	rs4997	C>A Trp64Arg	36774478	Intron 1	0.077	0.147
6	2215549	rs4994	(T>C)	36775507	Exon 1	0.081	0.174
7	12106148	rs4993	G>A	36775879	5′ UTR/Exon 1	1	1
8	8844728	rs802162	C>G	36778541	5′ Intergenic	0	0
9	2451694	rs13258937	C>T	36780439	5′ Intergenic	0	0

Markers #3 and #4 are known functional polymorphisms.
Physical locations are from the Celera Discovery system [CDS] database, September 2003. NCBI ID's are from the National Center for Biotechnology Information database, November 2003.
[a]Indicates tag SNPs for Caucasians.
[b]Indicates tag SNPs for African Americans.
Markers #7 and #8 are known functional polymorphisms.
Marker #6 is known functional polymorphism; marker #2 is monomorphic in Caucasians, and ##7–9 are monomorphic in both Caucasians and African Americans and then excluded from LD matrix (Figure 2c).

ADRB2 and *ADRB3* haplotype block regions, D' was generally >0.80 from one end of the region to the other. Average D' values within haplotype blocks in Caucasians and African Americans were, respectively, *ADRB1*: 0.98 and 0.84, *ADRB2:* 0.98 and 0.87, *ADRB3:* 1.00 and 0.74. Median D' values within the haplotypes blocks from both Caucasians and African Americans were high: *ADRB1:* 1.00 and 1.00, *ADRB2:* 1.00 and 1.00, and *ADRB3*: 1.00 and 0.93, indicating that most pairs of loci within these regions are in very high LD.

Haplotype frequencies for *ADRB1*, *ADRB2* and *ADRB3* in both populations are shown in Table 3a–c. For each population and haplotype block, 2–5 common (frequency $\geqslant$0.05) haplotypes accounted for most of the total: 88–100% of Caucasian and 88–96% of African-American haplotypes. For US Caucasians and African Americans, the numbers of common (frequency $\geqslant$0.05) haplotypes were: in *ADRB1*, 3 and 5; in *ADRB2,* 4; in *ADRB3,* 2 and 4, respectively. Population differences in haplotype frequencies are clearly illustrated in Figure 3a–c.

The marker panels we genotyped were sufficient to capture diversity in all blocks in the two populations we studied. We evaluated haplotype diversity within each block by successively subtracting SNPs from the haplotypes

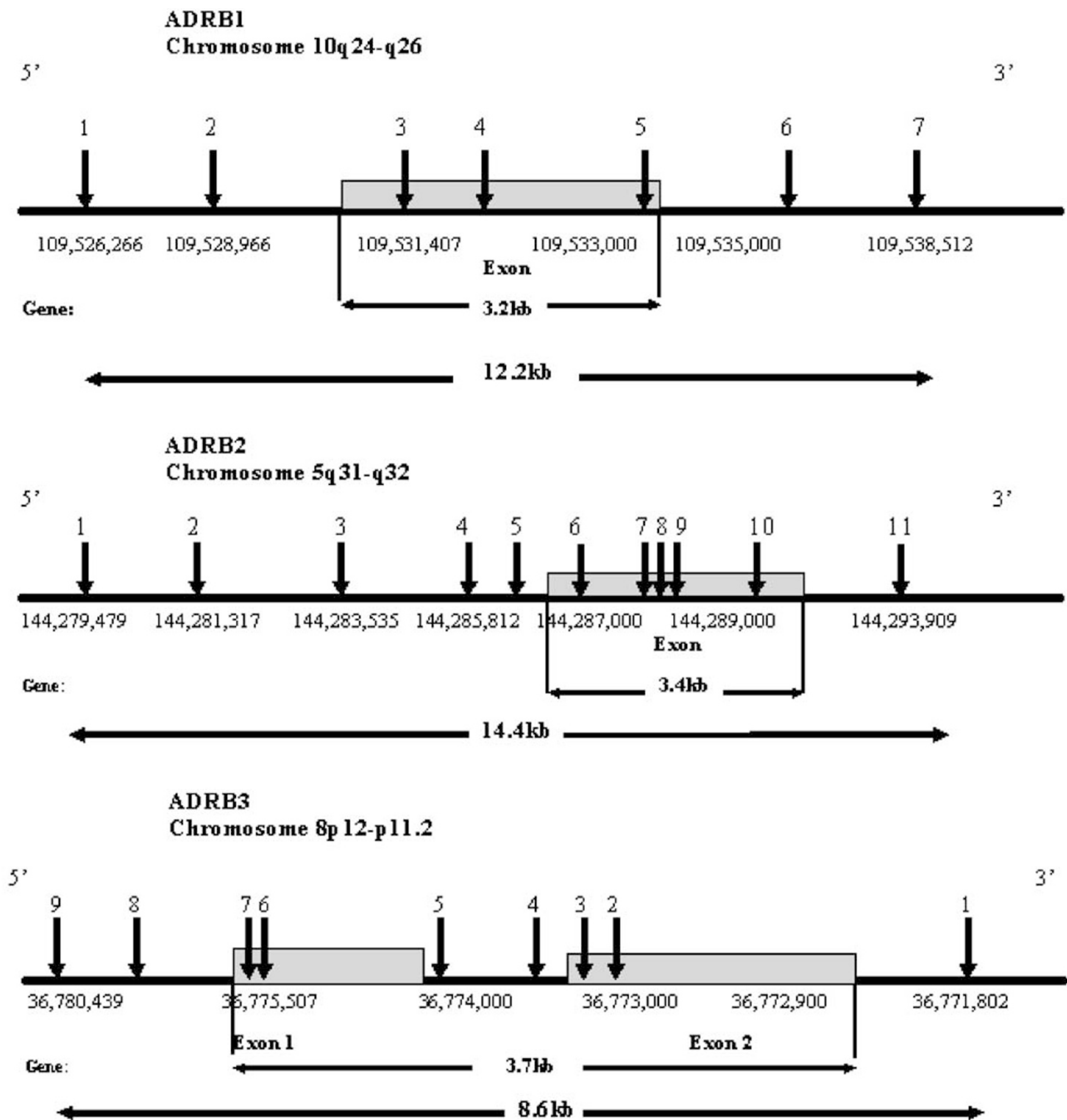

Figure 1 Locations of single-nucleotide polymorphisms genotyped in *ADRB1, ADRB2* and *ADRB3*. Coding exons are shown as solid blocks. Physical locations are from the Celera Discovery System [CDS] database, September 2003. **ADRB3* is transcribed in the reverse direction.

to evaluate the increment/decrement in diversity contributed by each SNP. SNPs were serially subtracted in that order that minimized the decrement in diversity at each step, and until only a single SNP (ie the SNP with the highest heterozygosity) remained. The chosen measure of diversity (haplotype frequencies and diplotype heterozygosity)

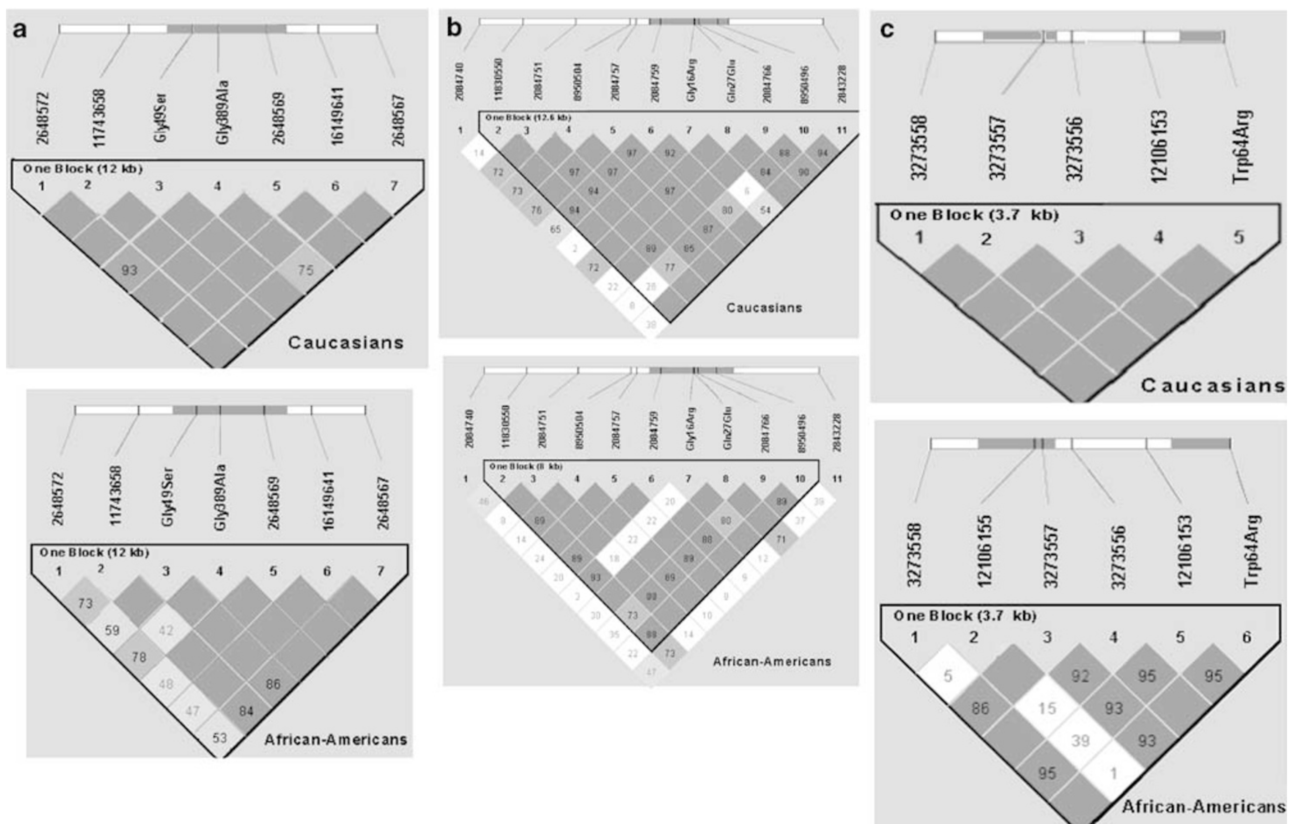

Figure 2 (**a–c**) Haplotype block organization of *ADRB1, ADRB2* and *ADRB3*. Each box represents % LD [*D*′] between pairs of markers, as generated by Haploview (Whitehead Institute for Biomedical Research, USA). *D*′ is color coded, red box indicating complete [1.00] *D*′ between locus pairs. **ADRB3* marker #2, monomorphic in Caucasians, is excluded from the Caucasian haplotype map, but included, for comparability to African Americans, in all haplotypes (see Table 3c).

was recalculated for each size SNP panel [n, $n-1$,…,1]. At some point for each haplotype block and for each population, adding or subtracting a SNP does not appreciably alter diversity, as shown in Figure 4, panels A-C. For *ADRB1*and *ADRB2*, haplotype diversity was highest in African Americans. A similar number of markers (2–4) was sufficient to capture maximum diversity in either population. This number represents an optimal panel, itself derived from the larger panel of SNP markers we genotyped. The minimum SNP set necessary to maximize haplotype diversity was also determined using SNPTagger.[37] The SNPs that constitute this minimal set are indicated in Table 2a–c.

For each β-AR gene, extensive amounts of resequencing have been performed and missense polymorphisms are known within each gene.[38–43] However, resequencing has been largely confined to the coding regions and to only a few populations. Although a complete inventory of common missense variants may be available in Caucasians and African Americans, unknown loci affecting function may be present, and some loci that are known may have unrecognized functional significance. Individual SNP loci provided some ability to capture information on the missense polymorphisms known at each gene (r^2 values ranged in Caucasians and African Americans, respectively: *ADRB1* Gly49Ser: 0.04–1.00 and 0.13–0.71, *ADRB1* Gly389Ala: 0.03–0.84 and 0.03–0.59, *ADRB2* Gly16Arg: 0.01–0.83 and 0.06–0.5, *ADRB2* Gln27Glu: 0.14–0.94 and 0.02–0.23, *ADRB3* Trp64Arg: 1.00 and 0.01–0.95, and as shown in Table 4a–c). Haplotypes enabled high sensitivity of detection of the missense substitutions (when a missense allele was present a particular haplotype(s) was present) and specificity of detection (when a haplotype(s) was present the missense allele was present). For each of the three β-AR genes, the haplotype was capable of capturing all or almost all the information provided by directly genotyping the missense loci, in either population (Table 5a, b). It is therefore likely that the SNP panels covering β-AR gene regions would capture information on unknown functional alleles. Certainly, genotyping of

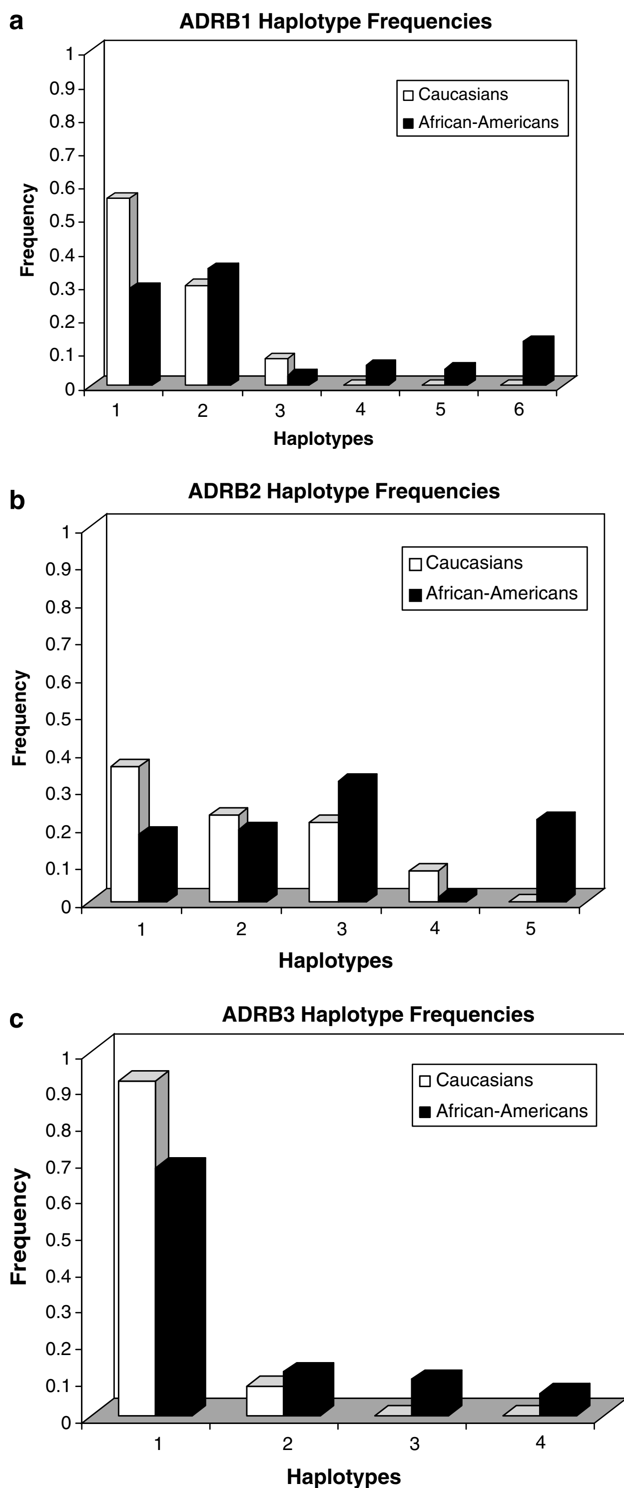

Figure 3 (**a**–**c**) Frequencies of common β-AR haplotypes in US Caucasians and African Americans.

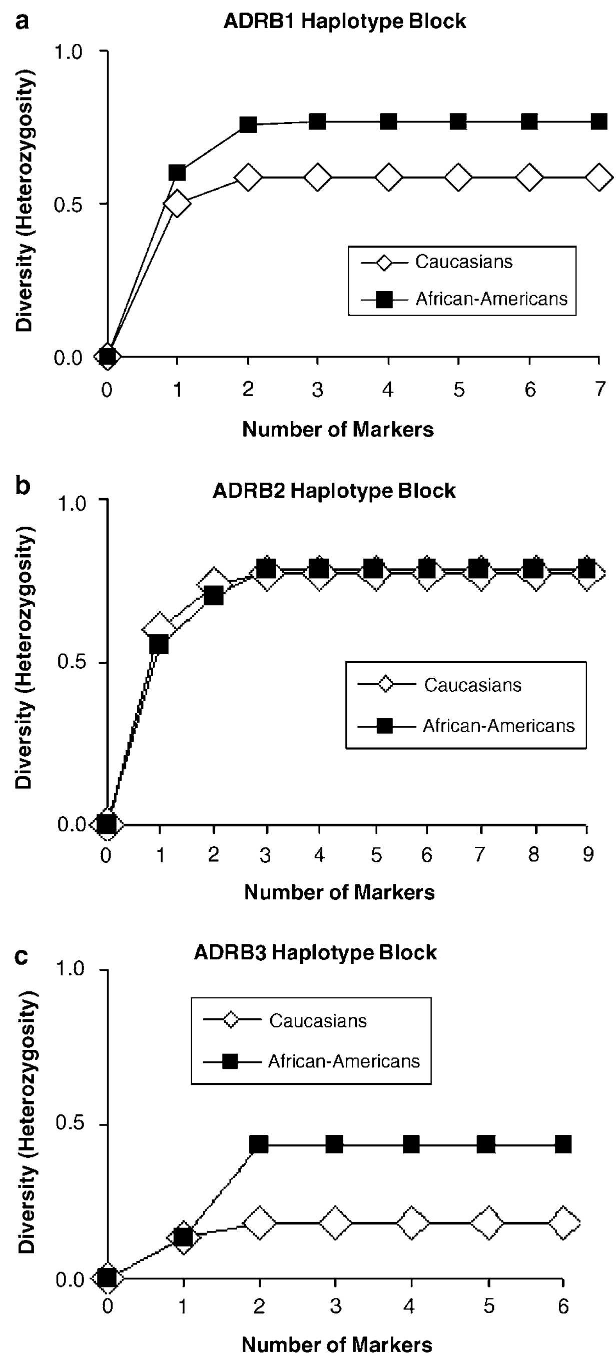

Figure 4 (**a**–**c**) Effect of successive subtraction/addition of SNPs on β-AR haplotype diversity in two populations. SNPs were successively subtracted from haplotypes in such a way as to minimize loss of diversity (diplotype heterozygosity, *Y*-axis). Panel (**a**) *ADRB1*, panel (**b**) *ADRB2* and panel (**c**) *ADRB3*. For each block, marker panels are sufficient to maximize diversity, and diversity can in fact be maximized with 2–4 optimal markers. For each haplotype panel, addition of the functional β-AR locus (or loci) yields no further increment in diversity.

polymorphisms that affect gene expression and/or function is highly important in association/linkage studies. However, there is a possibility that an unrecognized functional locus contributes to a phenotype. The focus of the haplotype-based approach to analyzing case–control populations has been to detect the effects of every functional locus, known or unknown.

For the β-AR genes, we have created multilocus SNP panels to define haplotype structure across each gene region. Each panel is sufficient to capture the signal of the moderately abundant missense alleles and unknown functional loci. The β-AR gene haplotype maps and marker panels provide a basis for future studies to investigate the role of genetic variation in physiology and pathophysiology related to β-AR function.

Table 3 Frequencies of haplotypes

# Common haplotypes	Frequencies	
	US Caucasians	*African Americans*
(a) *ADRB1* constructed from seven markers		
1 1222212 (GTACCGA)	0.56	0.29
2 2221212 (ATAGCGA)	0.30	0.35
3 1112121 (GGGCTAG)	0.08	0.03
4 1121212 (GGAGCGA)	0	0.06
5 1112212 (GGGCCGA)	0	0.05
6 1112211 (GGGCCGG)	0	0.13
(b) *ADRB2* constructed from nine markers		
1 222111212 (AAAGAGCAG)	0.36	0.18
2 111222112 (GTGAGAGAG)	0.23	0.19
3 211221121 (ATGAGGGGC)	0.21	0.32
4 111222111 (GTGAGAGAC)	0.08	0.01
5 222112112 (AAAGAAGAG)	0	0.22
(c) *ADRB3* constructed from six markers		
1 212211 (ACCCCT)	0.92	0.68
2 111122 (GCGTAC)	0.08	0.12
3 211211 (ACGCCT)	0	0.10
4 221211 (ATGCCT)	0	0.06

(1 = allele 1; 2 = allele 2).
Marker #3 is Gly49Ser (1 = Gly, 2 = Ser) and marker #4 is Gly389Ala (1 = Gly, 2 = Ala).
Marker #7 is Gly16Arg (1 = Gly, 2 = Arg) and marker #8 is Gln27Glu (1 = Gln, 2 = Glu).
Markers #1 and #11 are not part of the African-American haplotype block and thus were excluded.
Marker #6 is Trp64Arg (1 = Trp, 2 = Arg). Markers #7, #8 and #9 were monomorphic in both Caucasians and African Americans and were thus excluded.

Table 5 Effect of functional markers on haplotype diversity

Gene	*Variation*	*Marker frequency*	*Haplotype diversity with marker*	*Haplotype diversity without marker*	*Haplotype diversity without both markers*
(a) In Caucasians					
ADRB1	Gly49Ser	0.07	0.59	0.59	0.59
	Gly389Ala	0.34	0.59	0.59	0.59
ADRB2	Gly16Arg	0.34	0.77	0.77	0.77
	Gln27Glu	0.38	0.77	0.77	0.77
ADRB3	Trp64Arg	0.08	0.14	0.14	
(b) In African Americans					
ADRB1	Gly49Ser	0.26	0.78	0.78	0.77
	Gly389Ala	0.43	0.78	0.78	0.77
ADRB2	Gly16Arg	0.45	0.78	0.78	0.70
	Gln27Glu	0.19	0.78	0.78	0.70
ADRB3	Trp64Arg	0.17	0.51	0.51	

Table 4 R^2 values for functional markers *versus* noncoding SNPs

(a) For *ADRB1* haplotype block										
Caucasians	1	2	3	4	5	6	7			
Gly49Ser (Marker #3)	0.04	1.00	—	0.04	1.00	1.00	0.83			
Gly389Ala (Marker #4)	0.84	0.04	0.04	—	0.04	0.04	0.03			
African Americans										
Gly49Ser (Marker #3)	0.13	0.71	—	0.26	0.13	0.13	0.60			
Gly389Ala (Marker #4)	0.59	0.11	0.26	—	0.03	0.03	0.21			
(b) For *ADRB2* haplotype block										
Caucasians	2	3	4	5	6	7	8	9	10	11
Gly16Arg (Marker #7)	0.83	0.32	0.30	0.33	0.33	—	0.32	0.13	0.01	0.07
Gln27Glu (Marker #8)	0.38	0.96	0.94	0.92	0.91	0.32	—	0.23	0.27	0.14
African Americans	2	3	4	5	6	7	8	9	10	
Gly16Arg (Marker #7)	0.22	0.06	0.07	0.07	0.07	—	0.19	0.31	0.50	
Gln27Glu (Marker #8)	0.02	0.23	0.22	0.22	0.22	0.19	—	0.14	0.08	
(c) For *ADRB3* haplotype block										
Caucasians	1	2	3	4	5	6				
Trp64Arg (Marker #5)	1.00	1.00	1.00	1.00	—					
African Americans										
Trp64Arg (Marker #6)	0.94	0.01	0.33	0.95	0.85	—				

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