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ORIGINAL ARTICLE

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Haplotype-based analysis of alpha 2A, 2B, and 2C adrenergic receptor genes captures information on common functional loci at each gene

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Abstract The alpha 2-adrenergic receptors (α_2-AR) mediate physiological effects of epinephrine and norepinephrine. Three genes encode α_2-AR subtypes carrying common functional polymorphisms (*ADRA2A* Asn251Lys, *ADRA2B* Ins/Del301-303 and *ADRA2C* Ins/Del322-325). We genotyped these functional markers plus a panel of single nucleotide polymorphisms evenly spaced over the gene regions to identify gene haplotype block structure. A total of 24 markers were genotyped in 96 Caucasians and 96 African Americans. *ADRA2A* and *ADRA2B* each had a single haplotype block at least 11 and 16 kb in size, respectively, in both populations. *ADRA2C* had one haplotype block of 10 kb in Caucasians only. For the three genes, haplotype diversity and the number of common haplotypes were highest in African Americans, but a similar number of markers (3–6) per block was sufficient to capture maximum diversity in either population. For each of the three genes, the haplotype was capable of capturing the information content of the known functional locus even when that locus was not genotyped. The α_2-AR haplotype maps and marker panels are useful tools for genetic linkage studies to detect effects of known and unknown α_2-AR functional loci.

Keywords Single-nucleotide polymorphism · Insertion/deletion polymorphism · Linkage disequilibrium · Haplotype · Alpha 2-adrenergic receptors *ADRA2A* · *ADRA2B* · *ADRA2C*

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Introduction

Alpha 2-adrenergic receptors (α_2-AR) are widely distributed in the human central and peripheral nervous systems. They are cell surface G-protein-coupled receptors for the endogenous catecholamines, epinephrine and norepinephrine, mediating part of the diverse biological effects of these neurotransmitters. They are involved in the regulation of blood pressure by mediating contraction of vascular smooth muscle and induction of coronary vasoconstriction in humans (Civantos Calzada and Aleixandre de Artinano 2001; Comings et al. 2000). These receptors also modulate sedation, analgesia, insulin release, renal function, cognition, memory, and behavior (Berthelsen and Pettinger 1977; McGrath et al. 1989; Timmermans and van Zwieten 1981). Three distinct subtypes of α_2-AR—α2A, α2B, and α2C—have been identified in multiple mammalian species by molecular and pharmacological research. Because of the lack of subtype-selective agonists and antagonists, mice overexpressing, totally lacking (knockout), or expressing heavily modified α_2-AR subtypes have been generated to determine the specific functions of the three α_2-AR

subtypes (MacDonald et al. 1997). Each of the α_2-AR subtypes is specific in its distribution in tissues and cells, ontogenetic pattern, regulation, and physiological functions (Shishkina and Dygalo 2002).

Alpha 2A receptors are the predominant α_2-AR subtype in the central nervous system where they modulate sympathetic outflow and mediate the central antihypertensive action of the α_2-AR agonists clonidine and moxonidine (Gavras et al. 2001). The α2B subtype is the principal mediator of the hypertensive response to α_2-AR agonists, appears to play a role in salt-induced hypertension by eliciting a sympathoexcitatory response, and may be important in developmental processes (Kintsurashvili et al. 2003). The α2C subtype is involved in many central nervous system processes including the startle reflex, stress response, and locomotion. Both α2A and α2C are essential, as deletion of α2A and α2C receptors leads to cardiac hypertrophy and failure due to chronically enhanced catecholamine release (Hein 2001).

Physiologic functions controlled by different α_2-AR subtypes, including cardiovascular and other responses to α_2-AR agonists, are subject to interindividual variation in the human population. It can be speculated that some of the interindividual variation in responses is explained by genetic variation in the receptors producing changes in the amount or structure of the receptors. In support of a role for genetic variation, several physiologic parameters modulated by adrenergic function are heritable, e.g., blood pressure (Mathias et al. 2003), nociception (Lariviere et al. 2002), mood, and anxiety (Johansson et al. 2001).

Each α_2-AR subtype is encoded by a unique gene. Three genes (*ADRA2A*: hCG41806, *ADRA2B*: hCG37297, and *ADRA2C*: hCG1981539) are located on chromosomes 10q24-q26, 2p13-q13, and 4p16 respectively. They are all intronless and approximately 2.8–3.7 kb in length. For each α_2-AR subtype, sequence variations within the coding region of each gene that alter the structure of each α_2-AR protein have been identified in humans. These result in substitutions or deletions of amino acids in the third intracellular loops of each receptor. The consequences of each polymorphism for receptor signaling, as determined in transfected cells, include alterations in G-protein coupling, desensitization, and G-protein-receptor kinase-mediated phosphorylation (Small and Liggett 2001). The prevalences of the polymorphisms differ across ethnic populations. The three polymorphisms are each relatively abundant, and two are functional in vitro.

ADRA2A Asn251Lys is a functional amino acid substitution. Lys251 confers significantly increased agonist-promoted binding to Gi, leading to greater inhibition of adenylyl cyclase, activation of MAP kinase signaling, and stimulation of inositol phosphate accumulation (Small et al. 2000a). Lys251 has a frequency of 0.05 in African Americans compared with 0.004 in Caucasians, but is not associated with essential hypertension.

A polymorphism of *ADRA2B* consisting of a deletion of three glutamic acids (residues 301–303) from a glutamic acid repeat element in the third intracellular loop is more common in Caucasians (allele frequency 0.31) than African Americans (allele frequency 0.12) (Small et al. 2001). The presence of the del 301–303 allele leads to a small decrease in coupling efficiency resulting in reduced inhibition of adenylyl cyclase (Makaritsis et al. 1999). It has been associated with a reduced basal metabolic rate in obese subjects, with an increase in body weight among nondiabetic subjects, and modulation of autonomic nervous function in nondiabetic men (Sivenius et al. 2001, 2003). However, there is no evidence on the role of this *ADRA2B* variant in genetic susceptibility to essential hypertension mediated by *ADRA2B*.

In *ADRA2C*, six sequence variants include five synonymous substitutions (allele frequencies 0.006–0.25) and an in-frame 12-nucleic acid deletion encoding a receptor lacking Gly-Ala-Gly-Pro in the third intracellular loop at codons 322–325 (Feng et al. 2001; Small et al. 2000b). This deletion allele has frequencies of approximately 0.44 in African Americans and 0.035 in Caucasians (Feng et al. 2001). There is in vitro evidence that the deletion alters high-affinity agonist binding, indicating impaired formation of the agonist-receptor-G-protein complex (Small et al. 2000b). Since studies with α2C knockout mice and mice overexpressing α2C revealed changes in behavior and catecholaminergic function, such as locomotor activity in response to amphetamine, isolation-induced aggression paradigm or levels of dopamine, norepinephrine and serotonin (Kable et al. 2000), this functional variant may also be associated with effects on the same phenotypes in humans.

Each of the α_2-AR genes displays a functional polymorphism, but the currently known variants only contribute modestly to gene expression and/or function. Other functional loci may be present, including polymorphisms that are known but have not yet been recognized to be functional. Therefore, we have combined two genetic approaches—study of individual functional variants and haplotype analysis—to provide comprehensive coverage of the candidate gene for information content. In the present study, we develop a haplotype map for each of the three α_2-AR for two populations, American Caucasians and African Americans, by genotyping a panel of SNP markers and the known functional polymorphisms in these populations.

Materials and methods

Participants

A total of 192 unrelated subjects were genotyped, including 96 individuals from each of two populations: U.S. Caucasians and African Americans. Informed consent was obtained according to human research protocols approved by the human research committees

of the recruiting institutes: 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, November 2003) were used to select SNPs. 5′ nuclease assays (*vide infra*) could be designed for nine *ADRA2A*, eight *ADRA2B*, and seven *ADRA2C* SNPs and could be genotyped in highly accurate fashion. These panels of approximately equally spaced markers covered the entire genes plus 4–6 kb upstream and 4–6 kb downstream each gene.

Genomic DNA

Genomic DNA was extracted from lymphoblastoid cell lines and diluted to a concentration of 10 ng/μl. Aliquots of 1 μl aliquots were dried in 384-well plates. Genotyping was performed by the 5′ nuclease method (Shi et al. 1999) using fluorogenic allele-specific probes. Oligonucleotide primer and probe sets were designed based on gene sequence from the CDS, November 2003. Primers and detection probes for each locus in each gene are listed in Table 1.

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 endpoint 6-FAM or VIC fluorescence intensities at 508 nm 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.

Non-SNP markers

Two known functional deletion polymorphisms in *ADRA2B* and *ADRA2C* were genotyped by DNA fragment analysis on a capillary sequencer (ABI 3100). Forward and reverse primers were designed using Vector NTI Software (InforMax Inc., Bethesda, MD, USA); their sequences are shown in Table 1. To amplify DNA fragments, optimization was performed at varying annealing temperatures and magnesium chloride concentrations. The *ADRA2B* ins/del 20 μl reaction volume contained 100 ng genomic DNA, 2 μl 10× Buffer II (ABI), 1.5 mM $MgCl_2$, 20 ng of each primer, 0.2 mM dNTPs (Invitrogen), and 1 U Taq Gold Polymerase (ABI). The *ADRA2C* ins/del 20-μl reaction volume also contained 100 ng genomic DNA and 20 ng of each primer but 4 μl 5× buffer A (Invitrogen), 0.8 mM dNTPs (ABI), 0.5 μl Platinum Taq Polymerase (Invitrogen), and 2 μl DMSO. *ADRA2B* ins/del was amplified by a 12-min hot start at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at the optimal annealing temperature (65°C), 30 s at 72°C for elongation, and a final 10 min elongation at 72°C. *ADRA2C* ins/del was amplified by a 4-min hot start at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at the optimal annealing temperature (65°C), 30 s at 72°C for elongation, and a final 7-min elongation at 72°C. PCR was carried out with an ABI 9700. *ADRA2B* and *ADRA2C* amplicons were mixed together and with 3 μl of internal standard ROX 500 (ABI) and denatured at 95°C for 5 min. Data collected by ABI 3100 Genetic Analyzer were further analyzed by Genotyper V. 1.0.1 on the device.

Haplotype analysis

Haplotype frequencies were estimated using a Bayesian approach implemented with PHASE (Stephens et al. 2001). These frequencies closely agreed with results from a maximum likelihood method implemented via an expectation-maximization (EM) algorithm (Long et al. 1995). Haploview V. 2.0.2 (Whitehead Institute for Biomedical Research, USA) was used to produce LD matrices. Haplotype blocks were reconstructed using the pairs of markers with LD greater than 0.85 (Gabriel et al. 2002). SNPTagger (Ke and Cardon 2003) was used to determine the minimum SNP set that provides maximal haplotype diversity. Tag SNPs were identified by running the ''Fraction of haplotype patterns to be covered,'' being 0.85, using all the haplotypes produced by PHASE. The SNP sets we indicate is one possible alternative from among several that may be closely equivalent.

Results and discussion

Of a total of 24 markers in three α_2-AR genes, 23 were polymorphic both in Caucasians and African Americans. The functional *ADRA2A* SNP Asn251Lys was monomorphic in Caucasians. Dramatic interpopulation differences in allele frequencies were observed for most of the markers. Allele frequencies of all markers and their locations in the genes are shown in Table 2. The majority of the markers are located in the intergenic space upstream and downstream of each gene (Fig. 1 a–c). Functional nonsynonymous and one synonymous substitutions are located in the *ADRA2A* exon, and one marker is located in its 3′ UTR region. A functional

Table 1 Primer and probe sequences for 5′ nuclease genotyping

Number	Primers and probes	Sequences
Nine *ADRA2A* markers		
1	Assay on Demand 996421 (ABI, Ca)	
2	Assay on Demand 996423 (ABI, Ca)	
3	Forward primer	GCTATTCTTATCAGAGAGAACATGGAAA
	Reverse primer	CACCCCCATTCAGATGTACACA
	Allele 1 probe (FAM)	AGCATCCTTCATTAAG
	Allele 2 probe (VIC)	AGCATCCTTCAATAAG
4	Forward primer	CGGAGATAGGAGAAGGCTCTGTT
	Reverse primer	GGTCACGGCTGAGAAGCG
	Allele 1 probe (FAM)	CCTCCCGTCTCTT
	Allele 2 probe (VIC)	TCCTCCAGTCTCTTAG
5	Forward primer	GTGCCCGTTGCGTTCTG
	Reverse primer	TGGGAGTTGGCCATGCA
	Allele 1 probe (FAM)	CCGTCGGCCCCGAG
	Allele 2 probe (VIC)	CCGTCGGCCCGGAG
6	Forward primer	ATCTACCAGATCGCCAAGCGT
	Reverse primer	TGGGTGGGCAGCGGTT
	Allele 1 probe (FAM)	CCCAGACCCTTGGG
	Allele 2 probe (VIC)	CCCAGACCGTTGGG
7	Forward primer	GGACGCCGGCTGCA
	Reverse primer	GCCAGCACGAACGTGAAG
	Allele 1 probe (FAM)	CGGTTCTGCCGCCCG
	Allele 2 probe (VIC)	CGGTTCTGCCTCCCG
8	Forward primer	CCCCTTCCATTCCCAACTCT
	Reverse primer	GCAGGGCTGCCCTTAGC
	Allele 1 probe (FAM)	CTCTCTTTTTGAAGAAAA
	Allele 2 probe (VIC)	CTCTCTCTTTTTAAAGAAA
9	Forward primer	GGGATGTGGTGAGTTGCTAATCAA
	Reverse primer	GGATGGTCCTTGACACAAAGCA
	Allele 1 probe (FAM)	CAGAGGATCCCCTGTGC
	Allele 2 probe (VIC)	CAGAGGATCCACTGTGC
Eight *ADRA2B* markers		
1	Assay on Demand 180268 (ABI, Ca)	
2	Forward primer	GGTGCGAGCTGACATCCA
	Reverse primer	CTTGGGAAAGGCTGTTGTCAGA
	Allele 1 probe (FAM)	CTTCCAGCCCTGGCCT
	Allele 2 probe (VIC)	TTCCAGCGCTGGCCT
3	Forward primer	GGCCACTAAGTAGGGCAGGAA
	Reverse primer	TGTGGCTGGGTTTGAAAACAG
	Allele 1 probe (FAM)	TAGCCCCGGGTGGT
	Allele 2 probe (VIC)	TAGCCGCGGGTGG
4[a]	Forward primer (FAM)	GATACTGGGACCCGGGCCTT
	Reverse primer (ABD Tail)	CAGGAGCACCTGGCCACGTA
5	Forward primer	GGCACCTTGCAGTGCTT
	Reverse primer	GTTCCCCTTCTTCTTCAGCTACAG
	Allele 1 probe (FAM)	CTGGGCGCCATCT
	Allele 2 probe (VIC)	CCTGGGAGCCATCT
6	Forward primer	AGTCCCAGACCAGCAACAG
	Reverse primer	CCCACTCCCCTGCTATGTG
	Allele 1 probe (FAM)	CGGAGCGTTCCCAG
	Allele 2 probe (VIC)	CCGGAGCTTTCCCAG
7	Assay on Demand 7502922 (ABI, Ca)	
8	Assay on Demand 2089192 (ABI, Ca)	
Seven *ADRA2C* markers		
1	Forward primer	AGGCCCCAGGCTGAGA
	Reverse primer	GACCTTTCCCAGCCTTGGT
	Allele 1 probe (FAM)	ATTCTGAGGAGTGTGCA
	Allele 2 probe (VIC)	TCATTCTGAAGAGTGTGCA
2	Forward primer	AGTTCCTCAAGCCTAATTTCAAGGT
	Reverse primer	CCCTGGGCCATGCATAGG
	Allele 1 probe (FAM)	CCGGGCCCTCCCA
	Allele 2 probe (VIC)	CCCGGGTCCTCCCA
3	Forward primer	CAGCAGCATGTCAGCATGTT
	Reverse primer	CCAGGCTGAGGGCATCTG
	Allele 1 probe (FAM)	TGGGCCGCTCACT
	Allele 2 probe (VIC)	CTGGGCCACTCACT
4[a]	Forward primer (HEX)	AGACGGACGAGAGCAGCGCA
	Reverse primer (ABD Tail)	AGGCCTCGCGGCAGATGCCGTACA

Table 1 (Continued)

Number	Primers and probes	Sequences
5	Forward primer	TGCAGCCCTGCCTTTCC
	Reverse primer	AGCAGCAGTGTGAACAGTGT
	Allele 1 probe (FAM)	CACCGTCAGGCCCA
	Allele 2 probe (VIC)	CACCGTGAGGCCCA
6	Forward Primer	GTCAAGGCCGATGAGGACAT
	Reverse Primer	AGCTGGGCCAACAGTTCAG
	Allele 1 probe (FAM)	CCTCCCCCGACCAGA
	Allele 2 probe (VIC)	CCTCCCCCAACCAGA
7	Forward Primer	ATGGGACTCAGAGAGGTGGATT
	Reverse Primer	GGGTCTTATCACCAGCTGTGT
	Allele 1 probe (FAM)	AACCTGCCTGAGGTC
	Allele 2 probe (VIC)	ACCTGCGTGAGGTC

[a]Marker 4 is insertion/deletion (assay with labeled primers)

Table 2 Locations and allelic frequencies. Physical locations are from the Celera Discovery System (CDS) database, November 2003. NCBI IDs are from the National Center for Biotechnology Information database, November 2003

Number	SNP ID (CDS)	SNP ID (NCBI)	Variation	Position (CDS)	Location	Allelic frequency (for allele 2)	
						Caucasians	African Americans
Nine *ADRA2A* markers in 96 individuals from each of two populations							
1[a,b]	hCV996421	rs638019	G > A	106563427	5′ Intergenic	.26	.83
2[b]	hCV996423	rs491589	T > C	106566230	5′ Intergenic	.86	.77
3[b]	hCV3181570	rs521674	T > A	106567188	5′ Intergenic	.28	.76
4[b]	hCV7611986	rs1800763	C > A	106567590	5′ Intergenic	.03	.21
5	hCV7611979	rs1800544	C > G	106568101	5′ Intergenic	.27	.67
6[c]	hCV7611968	rs1800035	Lys 251 Asn (C > G)	106570150	Exon 1	1.00	.97
7[b]	hCV27473723	rs1800038	Arg 365 Arg (G > T)	106570940	Exon 1	.01	.08
8	hCV996424	rs553668	G > A	106571177	UTR 3′	.17	.31
9	hCV3181571	rs602618	C > A	106574687	3′ Intergenic	.72	.32
Eight *ADRA2B* markers in 96 individuals from each of two populations							
1[a]	hCV180268	rs13019188	G > A	91215007	3′ Intergenic	.44	.31
2[b]	hCV11510924	rs7561198	C > G	91215789	3′ Intergenic	.63	.77
3	hCV1841903	rs2252697	C > G	91218999	3′ Intergenic	.57	.70
4[d]	ADRA2B Ins/ Del	No rs	Del > nondel	91221466–91221474	Exon	.63	.79
5	hCV1841902	rs2229169	Gly 394 Gly (C > A)	91221526	Exon	.36	.27
6	hCV15919713	rs2692894	G > T	91225753	5′ Intergenic	.37	.27
7	hCV7502922	rs1168965	G > C	91228706	5′ Intergenic	.39	.22
8	hCV2089192	rs893173	T > C	91230594	5′ Intergenic	.43	.31
Seven *ADRA2C* markers in 96 individuals from each of two populations							
1&	hCV179118	rs7692883	G > A	3665266	5′ Intergenic	.12	.27
2&	hCV7667800	rs7667600	C > T	3668271	5′ Intergenic	.67	.33
3&	hCV179119	rs9790376	G > A	3668479	5′ Intergenic	.71	.39
4[e]&	ADRA2C Ins/ Del	No rs	Del > nondel	3672440–3672451	Exon	.94	.58
5&	hCV7666697	rs7678463	C > G	3673796	3′ Intergenic	.86	.42
6&	hCV7666695	rs13109333	G > A	3674571	3′ Intergenic	.83	.56
7	hCV499376	rs13112010	C > G	3675087	3′ Intergenic	.55	.20

[a] Indicates tag SNPs for Caucasians
[b] Indicates tag SNPs for African Americans
[c] Marker 6 is a known functional polymorphism
[d] Marker 4 is a known functional polymorphism
[e] Marker 4 is a known functional polymorphism

insertion/deletion polymorphism and one synonymous substitution are located in the *ADRA2B* exon. In *ADRA2C*, the functional insertion/deletion polymorphism is located in the coding region (exon). All genotype frequencies conformed to Hardy–Weinberg equilibrium.

Within the *ADRA2A* and *ADRA2B* regions, a single conserved haplotype block 11 and 16 kb in size, respectively, spanned each gene in both populations (Fig. 2 a,b) and the block boundaries extend beyond the region we have evaluated. The *ADRA2C* region had one haplotype block of 10 kb in Caucasians. In African Americans, no haplotype block was identified since only the first and last SNPs were in strong linkage disequilibrium (LD) with all other markers (Fig. 2 c). One possible reason for this could be excessive recombination in this population that might happen particularly because of the physical location of *ADRA2C* at the very top of chromosome 4. Isolated nucleotide substitutions occurring within nonrecombined blocks can also

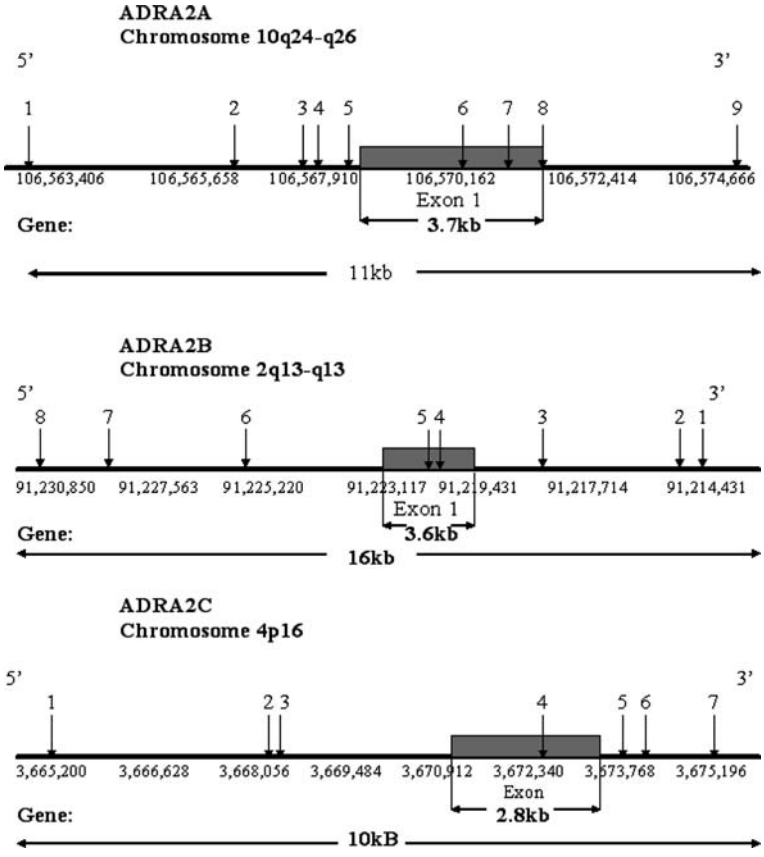

Fig. 1a–c Location of single-nucleotide polymorphisms genotyped in *ADRA2A*, *ADRA2B*, and *ADRA2C*. Coding exons are shown as solid blocks. Physical locations are from the Celera Discovery System (CDS) database, November 2003. *ADR2B* is transcribed in reverse orientation

contribute to the lack of LD in the region. Finally, recent studies reveal only a partial fit to the fundamentally ''block-like'' structure of the human genome (Wall and Pritchard 2003a, 2003b), and some regions including *ADRA2C* may not conform this model. Since the *ADRA2C* panel was of high marker density (seven markers across 10 kb involving 2.8 kb of the actual gene sequence), no improvement in the definition of haplotype block structure could be expected by expanding the population size (Wall and Pritchard 2003a).

Definition of haplotype blocks and block boundaries is inexact. 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 that might have originated from this cause. In the *ADRA2A*, *ADRA2B*, and *ADRA2C* haplotype block regions, D' was generally >0.85 from one end of the region to the other. Average D' values within haplotype blocks in Caucasians and Africa -Americans were, respectively, *ADRA2A*: 0.97 and 0.91, *ADRA2B:* 1.00 and 0.99, and *ADRA2C:* 0.83 and 0.56. Median D' values within the haplotype blocks from both Caucasians and African Americans were high: *ADRA2A:* 1.00 and 1.00, *ADRA2B:* 1.00 and 1.00, and *ADRA2C:* 0.88 and 0.57, indicating that most pairs of loci within these regions are in very high LD.

Haplotype frequencies for *ADRA2A* and *ADRA2B* in both populations are shown in Table 3. For each population and haplotype block, two to seven common (frequency ≥0.05) haplotypes accounted for most of the total: 78–95% of Caucasian and 73–89% of African American haplotypes. For Caucasians and African Americans, the numbers of common (frequency ≥0.05) haplotypes were in *ADRA2A*, 3 and 7; in *ADRA2B*, 2 and 2; and in *ADRA2C*, 4 and 8, respectively.

An important aspect of understanding the level of genetic information content within and between haplotype blocks is haplotype diversity (informativeness). For each α_2-AR gene haplotype block, a panel of markers sufficient to maximize genetic information content was available to address this issue. We evaluated haplotype diversity within each block by successively subtracting SNPs to the haplotypes 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 (i.e., the SNP with the highest heterozygosity) remained. The chosen measure of diversity (haplotype frequencies and diplotype heterozygosity) was recalculated for each SNP panel size (n, $n-1...1$). At some point for each haplotype block and for each population, adding or subtracting an SNP does not appreciably alter diversity, as shown in Fig. 3 a–c. For *ADRA2A* and *ADRA2C*, haplotype diversity was highest in African Americans. A similar number of markers (three to five) was necessary to capture maximum diversity in either population (with the exception of the *ADRA2A*

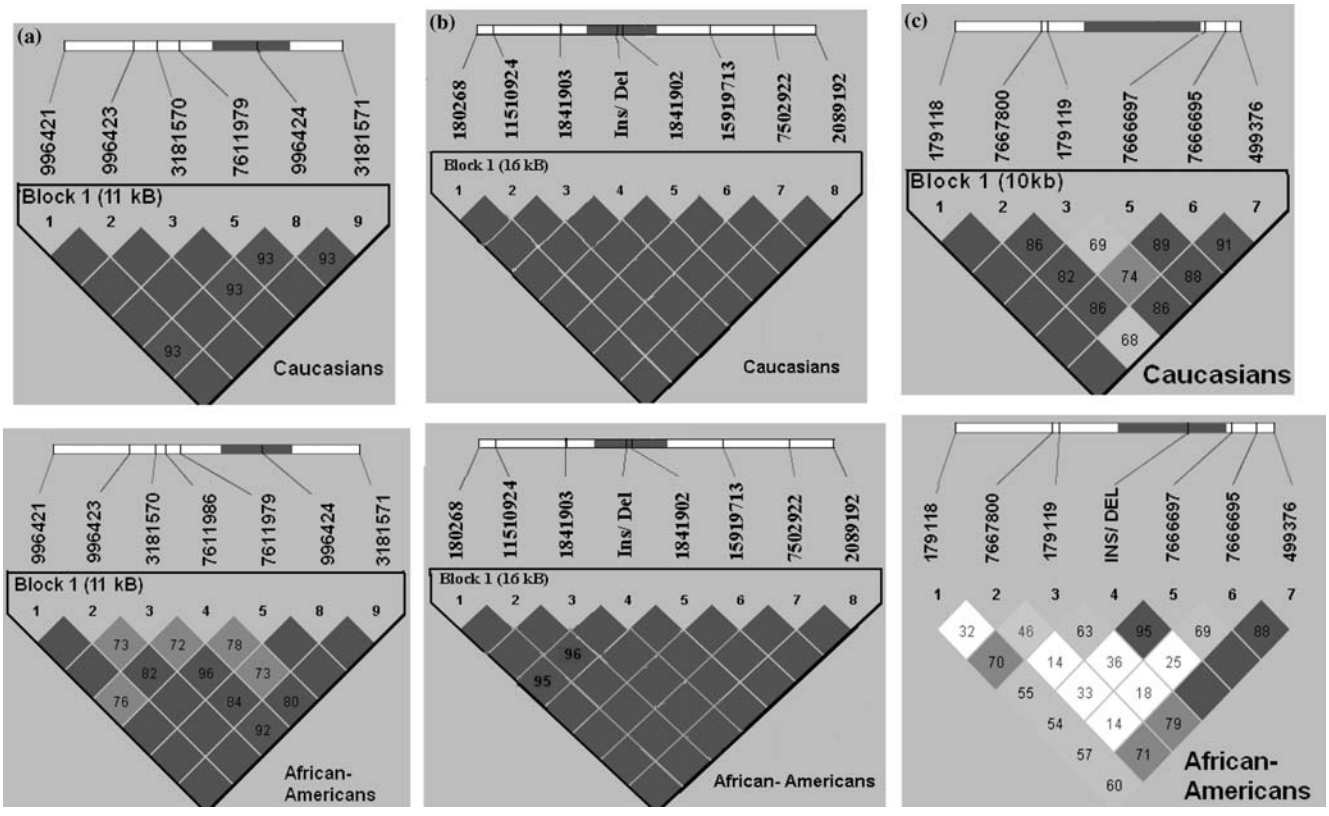

Fig. 2a–c Haplotype block organization of *ADRA2A, ADRA2B*, and *ADRA2C*. Each box represents percent of linkage disequilibrium (LD) (*D*') between pairs of markers, as generated by Haploview (Whitehead Institute for Biomedical Research, USA). *D*' is color coded, the *black box* indicating complete (1.00) *D*' between locus pairs. *ADRA2A* and *ADRA2C* LD matrices were estimated without low frequency markers

haplotype block, which required six markers to capture maximum diversity). This number represents an optimal panel, itself derived from the larger panel of SNP markers we genotyped. The SNPs that constitute the minimal set necessary to maximize haplotype diversity (85% of haplotypes covered) are indicated in Table 2.

For each α_2-AR gene, a known functional polymorphism was contained within the haplotype block. Within each block, haplotypes enabled high sensitivity of detection of the functional locus (when a functional allele was present, the particular haplotype(s) was present) and specificity of detection (when the haplotype(s) was present the functional allele was present). For each of the three α_2-AR genes, the haplotype was capable of capturing all or almost all the information provided by directly genotyping the functional locus in either population (Table 4). These SNP panels covering α_2-AR gene regions reliably capture haplotype diversity in different populations even when not including known functional alleles. Certainly, genotyping of 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.

Table 3 Frequencies of haplotypes

Number	Common haplotypes	Frequencies	
		Caucasians	African Americans
In *ADRA2A* constructed from nine markers (1 = allele 1; 2 = allele 2)			
1	121112112	0.72	0.16
2	212122121	0.15	0.21
3	222122111	0.08	0.18
4	222222111	0.03	0.17
5	222122221	0.00	0.07
6	222112112	0.00	0.05
7	221112112	0.00	0.05
8	222111112	0.00	0.03
In *ADRA2B* constructed from eight markers (1 = allele 1; 2 = allele 2)			
1	12221111	0.56	0.67
2	21112222	0.39	0.22
3	22121112	0.03	0.03
In *ADRA2C* constructed from seven markers (1 = allele 1; 2 = allele 2)			
1	1222222	0.49	0.15
2	1112221	0.11	0.13
3	2222221	0.10	0.02
4	1112111	0.08	0.07
5	1211111	0.02	0.07
6	1121111	0.03	0.06
7	1111111	0.01	0.05
8	2111121	0.00	0.14
9	1122111	0.00	0.06

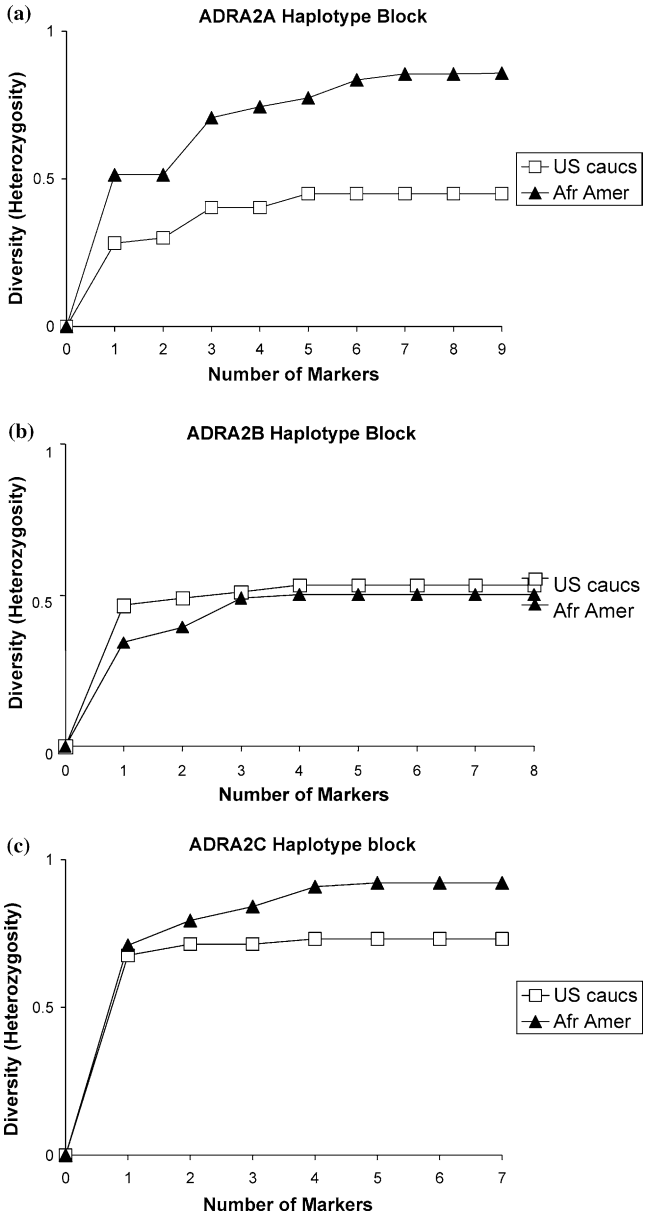

Fig. 3a–c Effect of successive subtraction/addition of SNPs on α-AR haplotype diversity in two populations. SNPs were successively subtracted from haplotypes in such a way as to minimize loss of diversity (diplotype heterozygosity, *Y* axis]. For each block, marker panels are sufficient to maximize diversity, and diversity can be generally maximized with three to five optimal markers. For each haplotype panel, addition of the functional α-AR locus yields no further increment in diversity

Some functional polymorphisms have low frequencies in certain populations. For example, *ADRA2A* Asn251-Lys is relatively common in African Americans (allele frequency 0.05) but only has a frequency of 0.004 in Caucasians, making it uninformative in studies limited to a small number of individuals. The *ADRA2A* SNP panel developed here includes eight markers in addition to Asn251Lys. When Asn251Lys is excluded, there is essentially no loss of *ADRA2A* information content in either African Americans or Caucasians (Table 4).

Table 4 Effect of functional marker on haplotype diversity

Variation	Marker frequency	Haplotype diversity with marker	Haplotype diversity without marker
In Caucasians			
ADRA2A (Asn 251)	0.00	0.46	0.46
ADRA2B Del	0.37	0.53	0.53
ADRA2C Del	0.06	0.73	0.73
In African Americans			
ADRA2A (Asn 251)	0.03	0.853	0.851
ADRA2B Del	0.21	0.51	0.51
ADRA2C Del	0.42	0.92	0.91

For the α_2-AR genes, we created multilocus SNP panels to define a specific LD structure across each gene region. Each panel is sufficient to capture the signal of the moderately abundant known functional locus at each gene, and these panels should also be informative for unknown functional loci. By using a comprehensive haplotype-based approach for the development of α_2-AR gene haplotype maps and marker panels, our study provides the basis for future studies to investigate the role of genetic risk factors associated with known and unknown functional loci in pathophysiological conditions linked to alpha 2-adrenergic receptor function.

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