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Diverse Evolutionary Histories for β-adrenoreceptor Genes in Humans

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In humans, three genes—*ADRB1*, *ADRB2* and *ADRB3*—encode β-adrenoreceptors (ADRB); these molecules mediate the action of catecholamines in multiple tissues and play pivotal roles in cardiovascular, respiratory, metabolic, and immunological functions. Genetic variants in *ADRB* genes have been associated with widespread diseases and conditions, but inconsistent results have often been obtained. Here, we addressed the recent evolutionary history of *ADRB* genes in human populations. Although *ADRB1* is neutrally evolving, most tests rejected neutral evolution for *ADRB2* in European, African, and Asian population samples. Analysis of inferred haplotypes for *ADRB2* revealed three major clades with a coalescence time of 1–1.5 million years, suggesting that the gene is either subjected to balancing selection or undergoing a selective sweep. Haplotype analysis also revealed ethnicity-specific differences. Additionally, we observed significant deviations from Hardy-Weinberg equilibrium (HWE) for *ADRB2* genotypes in distinct European cohorts; HWE deviation depends on sex (only females are in disequilibrium), and genotypes displaying maximum and minimum relative fitness differ across population samples, suggesting a complex situation possibly involving epistasis or maternal selection. Overall, our data indicate that future association studies involving *ADRB2* will benefit from taking into account ethnicity-specific haplotype distributions and sex-based effects. With respect to *ADRB3*, our data indicate that the gene has been subjected to a selective sweep in African populations, the Trp64 variant possibly representing the selection target. Given the previous association of the ancestral *ADRB3* Arg64 allele with obesity and type 2 diabetes, dietary adaptations might represent the underlying selective force.

Introduction

Adrenergic receptors are G protein-coupled molecules that mediate the action of catecholamines in multiple tissues. In particular, they are an integral part of the sympathetic nervous system and play pivotal roles in cardiovascular, respiratory, metabolic, and immunological functions.

Adrenergic receptors have been subdivided into two major types, α and β, on the basis of agonist-mediated responses, with subsequent classification into subtypes based on differential tissue localization (reviewed in [1]). As far as members of the β type (ADRB) are concerned, three distinct receptors have been identified in humans: β1, β2 and β3, all encoded by small intronless or single-intron genes located on chromosomes 10, 5 and 8, respectively.

ADRBs have been the subjects of intensive investigations as a result of their possible role in the pathophysiology of widespread conditions such as insulin resistance, obesity (MIM 601665), asthma (MIM 600807), and cardiovascular disorders.[2–6] Also, ADRBs are targets of many commonly used drugs; thus, the identification and analysis of functional variants is extremely relevant to pharamacogenetic studies. As a consequence, commonly occurring polymorphisms have been searched for and studied in *ADRB* genes and particular focus has been placed on nonsynonymous SNPs. A common R389G variant in *ADRB1* (MIM 109630) has been shown[7] to influence the receptor's coupling properties; the association of this SNP with obesity is controversial,[8,9] but experiments in transgenic mice have indicated that the R389 allele predisposes to heart failure.[10] Of four relatively common nonsynonymous SNPs in *ADRB2* (MIM 109690), three are functional in vitro: Gly16 leads to enhanced agonist-mediated down-regulation,[11] Glu27 reduces such regulation,[11] and Ile64 shows[12] impaired agonist binding and decreased adenyl cyclase activity. Moreover, additional functional variants have been identified in the promoter region of *ADRB2*.[13] The phenotypic effect of coding SNPs has been investigated in many studies; associations of Gly16 and Glu27 with asthma and obesity, respectively, have been reported, although not always validated in independent studies (reviewed in [14]). Similarly, contradictory results have been obtained among studies aiming at correlating genetic variants in *ADRB2* with hypertension (reviewed in [15]). In addition, conditions such as autism (MIM 209850),[16,17] preterm delivery,[18–21] cerebral palsy,[22] parasitic infection,[23] rheumatoid arthritis (MIM 180300),[24,25] and temporomandibular joint disorder[26] have been associated with *ADRB2* polymorphisms. With respect to *ADRB3* (MIM 109691), a low-frequency ancestral allele has been associated with obesity and type 2 diabetes (MIM 125853) in some but not all populations (reviewed in [27]).

Therefore, in analogy to many other cases, studies aiming at associating specific variants with common diseases

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have often been inconsistent with one another; in general, the lack of consistency among association studies is thought to be due to both false-positive and false-negative results rather than to variability in association for populations with different ethnic origins.[28] Whatever the reason, a clear picture of whether *ADRB* variants influence disease susceptibility is still missing.

Further insight into the genetic basis of common diseases can be obtained through molecular evolutionary studies of candidate loci; indeed, inference about selection models operating on specific gene regions implicitly implies the presence of functional variants with some effect on fitness (at some time in human history). Moreover, it has recently been proposed (reviewed in [29,30]) that the shift of environment and lifestyle that has been associated with two extremely relevant transitions, the out-of-Africa migration and the development of agricultural methods, might have rendered maladaptive genetic variants that had been selected for in earlier stages of human history. This hypothesis has been explicitly formulated for a few common diseases, such as obesity, hypertension, and asthma. On these bases, we set out to verify whether the neutral model of evolution applies to *ADRB* genes in humans.

Material and Methods

DNA Samples, Sequencing, and Genotyping

Human genomic DNA for East Asians (EAS), Australian Aborigines (AUA), and native South Americans (NSA) was obtained from either the Coriell Institute for Medical Research or the European Collection of Cell Cultures. For population genetics analyses, the 6 kb genomic portion encompassing *ADRB2* coding and promoter regions was PCR amplified in overlapping fragments. PCR products were treated with ExoSAP-IT (USB Corporation, Cleveland, OH, USA), directly sequenced on both strands with a Big Dye Terminator Sequencing Kit (v3.1 Applied Biosystems), and run on an Applied Biosystems ABI 3130 XL Genetic Analyzer (Applied Biosystems). Sequences were assembled with AutoAssembler version 1.4.0 (Applied Biosystems) and inspected manually by two distinct operators, and singletons were reamplified and resequenced. Primer sequences are available upon request. The number of subjects resequenced was as follows: AUA, 12; NSA, 24; EAS, 25.

For the study of haplotype and genotype frequencies in an Italian population sample, rs1042714 (Arg16Gly) and rs1042713 (Gln27Glu) were genotyped in 654 Italian individuals. Genomic DNA from these subjects was obtained from different sources; in particular, 263 subjects were collected and genotyped as previously described,[31] and the remaining individuals were either provided by the Telethon Bank of DNA, Nerve and Muscle Tissues (no. GTF02008), located at the Department of Neurological Sciences, IRCCS Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena Foundation, Milan, or recruited as volunteers at the Scientific Institute IRCCS E.Medea, after informed consent was obtained. Genotyping for these subjects was performed by direct sequencing of the gene region encompassing rs1042714 and rs1042713, with the use of the same procedures described above. The study was approved by the Ethical Committee of the Scientific Institute IRCCS E. Medea-Associazione La Nostra Famiglia, in agreement with Italian standards.

Data Retrieval and Haplotype Construction

Genotype data for two population samples, one of African and one of European ancestry, were retrieved from the SeattleSNPs website. In particular, *ADRB2* and *ADRB3* have been resequenced in 24 Yoruba (YRI) and 24 Utah residents with ancestry from northern and western Europe. Data for *ADRB1* refer to 24 African Americans (AA) and 23 individuals with European ancestry (20 Utah residents with ancestry from northern and western Europe and three French individuals). Populations with European ancestry are hereafter referred to as EU. The nucleotide positions for all analyzed genes correspond to those of SeattleSNPs, which in turn derive from the following GenBank accession numbers: *ADRB1*, AY567837; *ADRB2*, DQ094845; *ADRB3*, DQ104441.

Genotype data for 238 resequenced human genes were derived from the National Institute of Environmental Health Sciences (NIEHS) SNPs Program website. In particular, we selected genes that had been resequenced in genotype samples of populations of defined ethnicity, including AA, EU, YRI, and EAS (NIEHS panel 2). Data from the AA and EU samples were used as a comparison for *ADRB1*, which has been resequenced in these same populations. Similarly, for *ADRB2* and *ADRB3*, the comparison was performed with YRI and EU samples.

Haplotypes were inferred with PHASE version 2.1,[32,33] a program for reconstructing haplotypes from unrelated genotype data through a Bayesian statistical method. Inferred haplotypes for all individuals used in this study are available in the Supplemental Data (Tables S1 and S2, available online).

Statistical Analysis

Tajima's D statistic,[34] Fu and Li's D* and F*[35] statistics, as well as diversity parameters θ_W[36] and π[37] and Fay and Wu's H,[38] were calculated with libsequence,[39] a C++ class library providing an object-oriented framework for the analysis of molecular population-genetic data. The scaled per-nucleotide recombination parameter ($R = 4N_e r$) was estimated with MaxDip.[40]

Calibrated coalescent simulations were performed with the *cosi* package[41] and its best-fit parameters for YRI, AA, EU, and Asian populations with 10000 iterations.

A composite-likelihood-ratio test and coalescent simulations under a selective sweep regime were performed with the *clsw* and *ssw* programs, kindly provided by Yuseob Kim.

The F_{ST} statistic[42] estimates genetic differentiation among populations and was calculated as previously proposed.[43]

The multilocus HKA test was performed with the HKA software distributed by Jody Hey. Sixteen reference loci were randomly selected among NIEHS loci that are shorter than 20 kb and have been resequenced in genotype samples of the three populations (YRI, EU, and EAS; panel 2). The only criterion was that Tajima's D did not suggest the action of natural selection (i.e., D_T is higher than the 5th and lower than the 95th percentiles in the distribution of NIEHS genes). The reference set was accounted for by the following genes: *VNN3* (MIM 606592), *PLA2G2D* (MIM 605630), *MB* (MIM 160000), *MAD2L2* (MIM 604094), *HRAS* (MIM 190020), *CYP17A1* (MIM 609300), *ATOX1* (MIM 602270), *BNIP3* (MIM 603293), *CDC20* (MIM 603618), *NGB* (MIM 605304), *TUBA1* (MIM 191110), *MT3* (MIM 139255), *NUDT1* (MIM 600312), *PRDX5* (MIM 606583), *RETN* (MIM 605565), and *JUND* (MIM 165162). For each locus, 1000 coalescent simulations were performed with the *cosi* package[41] and only neutrally evolving sites were considered.

Median-joining networks for the inference of haplotype genealogy were constructed with NETWORK 4.5.[44] Estimate of the time to the most recent common ancestor (TMRCA) was obtained

Table 1. Summary Statistics for ADRB Genes

Gene	L[a]	Pop.	N[b]	S[c]	θ_W[d] Value	θ_W[d] Rank[e]	π[d] Value	π[d] Rank[e]	Tajima's D Value	Tajima's D p[f]	Fu & Li's D Value	Fu & Li's D p[f]	Fu & Li's F Value	Fu & Li's F p[f]
ADRB1	9.4	AA	48	35	8.73	0.50	5.75	0.31	−1.16	0.17	0.72	0.037	0.02	0.16
		EU	46	22	5.54	0.53	7.39	0.69	1.09	0.099	1.12	0.059	1.33	0.048
ADRB2	6	YRI	48	35	13.65	0.89	17.86	0.98	1.05	0.0066	−0.29	0.34	0.26	0.12
		EU	46	27	10.63	0.93	19.17	0.98	2.68	0.0003	1.32	0.031	2.20	0.0012
ADRB3	7.4	YRI	48	23	7.96	0.42	3.47	0.11	−1.84	0.048	−1.75	0.12	−2.15	0.060
		EU	46	8	2.80	0.14	1.59	0.09	−1.20	0.049	−1.62	0.090	−1.76	0.064

[a] Length of the analyzed region (from the most 5′ resequenced nucleotide to the end of the 3′ UTR).
[b] Sample size (chromosomes).
[c] Number of segregating sites.
[d] Nucleotide diversity indexes ($\times 10^{-4}$).
[e] Percentile rank relative to the distribution of 238 5 kb windows.
[f] p values obtained from coalescent simulations.

via a phylogeny-based approach implemented in NETWORK, with the use of a mutation rate based on the number of fixed differences between human and chimpanzee and the assumption of a separation time from humans of 6 MY ago.[45] A second TMRCA estimate was derived from application of a maximum-likelihood coalescent method implemented in GENETREE.[46,47] Again, the mutation rate μ was obtained on the basis of the divergence between human and chimpanzee and under the assumption of a generation time of 25 yrs. Using this μ and θ maximum likelihood (θ_{ML}), we estimated the effective population-size parameter (Ne). With these assumptions, the coalescence time, scaled in 2Ne units, was converted into years. For the coalescence process, 10^6 simulations were performed.

All calculations were performed in the R environment.[48]

Results

Nucleotide Diversity and Neutrality Tests for *ADRB* Genes

The three *ADRB* genes have been included in the Seattle SNPs variation-discovery resource (SeattleSNPs, NHLBI Program for Genomic Applications) and have been fully resequenced in samples of two populations with African (either YRI or AA) and European origin (EU).

We exploited the availability of these data to calculate nucleotide-diversity parameters for the three genes in these population samples; in particular, all the analyses reported below have been performed on gene regions extending from the most 5′ resequenced nucleotide to the 3′ UTR end.

Nucleotide diversity was assessed through two indexes: θ_W,[36] an estimate of the expected per-site heterozigosity, and π,[37] the average number of pairwise sequence nucleotide differences. Under neutral evolution, values of θ_W and π are roughly equal. The Tajima's D statistic (D_T,[34]) is commonly used to evaluate their difference and, therefore, departure from neutrality. Positive values of D_T indicate an excess of intermediate-frequency variants and are evidence of balancing selection, and negative D_T values indicate either purifying selection or a high representation of rare variants as a result of a selective sweep. Fu and Li's F and D (as well as F* and D*[35]) statistics are also based on SNP frequency spectra and differ from Tajima's D in that they also take into account whether mutations occur in external or internal branches of a genealogy. Because population history, in addition to selective processes, is known to affect frequency spectra and, therefore, all related statistics, we performed coalescent simulations by using a population genetics model that incorporates demographic scenarios.[41] Moreover, to the same aim, we exploited the conundrum whereby selection acts on a single locus while demography affects the whole genome, calculating diversity parameters and test statistics for a set of 238 genes resequenced by the NIEHS program; in particular, a 5 kb window was randomly selected for each gene (see Material and Methods).

Data for *ADRB* genes are reported in Table 1 and indicate that no departure from neutrality is observed for *ADRB1*. Conversely, significantly positive values of D_T and Fu and Li's D and F were obtained for *ADRB2* in Europeans. In Africans, the presence of nine singletons in *ADRB2* yielded low values for Fu and Li's statistics but D_T ranked highly when compared to the distribution of 238 resequenced genes in African populations (Table 2). Moreover, both θ_W and π results were higher for *ADRB2* than for most NIEHS genes (Table 1).

Finally, analysis of *ADRB3* revealed negative statistics (with marginally significant p values), as well as low levels of nucleotide variation, in YRI and EU (Table 1).

These findings prompted us to further investigate the evolutionary history of *ADRB2* and *ADRB3*.

The Signature of Selection at *ADRB2*

As a first step, we wished to verify whether rejection of the neutral model was also verified for other human population samples. We therefore resequenced the 6 kb encompassing *ADRB2* coding and promoter regions in three additional populations: EAS, NSA, and AUA. When model-fitting parameters were available (i.e., for EAS), we applied

Table 2. Summary Statistics for *ADRB2* in Five Human Populations

			Tajima's D			Fu & Li's D*			Fu & Li's F*		
Pop.	N[a]	S[b]	Value	p[c]	Rank[d]	Value	p[c]	Rank[d]	Value	p[c]	Rank[d]
YRI	48	35	1.05	0.0066	0.99	−0.22	0.34	0.59	0.27	0.11	0.82
EU	46	27	2.68	0.0003	0.99	0.93	0.083	0.91	1.83	0.0043	0.99
EAS	50	30	1.59	0.038	0.94	1.56	0.0052	0.99	1.87	0.0053	0.99
NSA	48	22	1.33	0.047	n.a.[e]	1.38	0.035	n.a.[e]	1.61	0.020	n.a.[e]
AUA	24	26	0.69	0.18	n.a.[e]	0.75	0.18	n.a.[e]	0.85	0.17	n.a.[e]

[a] Sample size (chromosomes).
[b] Number of segregating sites.
[c] p values obtained from coalescent simulations.
[d] Percentile rank relative to the distribution of 5 kb windows.
[e] Not available.

a population genetics model[41] for coalescent simulations; in the case of AUA and NSA, the standard neutral model was used. Nucleotide diversity parameters and neutrality tests are shown in Table 2 and indicate that, in analogy to EU, one or more statistics rejected neutrality for all population samples, excluding AUA. In agreement with these data, both D_T and Fu and Li's F* and D* display a high percentile rank in the distribution of NIEHS genes for EU and EAS (Table 2).

Under neutral evolution, the amount of within-species diversity is predicted to correlate with levels of between-species divergence, because both depend on the neutral mutation rate.[49] We performed a multilocus HKA test by using the HKA software, which allows estimation of statistical significance through coalescent simulations (see Material and Methods). As above, the latter were performed with the use of a previously described demographic model;[41] due to the lack of a demographic model, the test was not performed for AUA and NSA. Significant results were obtained for EU, YRI, and EAS (Table 3), and in all cases, the test of maximum cell value[50] indicated *ADRB2* as an outlier, its removal from the gene set yielding nonsignificant tests (Table 3).

Next, we wished to analyze the structure of *ADRB2* inferred haplotypes. Construction of a median-joining network[44] (Figure 1) revealed the presence of three major clades (haplogroups A, B, and C, hereafter referred to as H_A, H_B, and H_C) separated by long branch lengths, each containing common inferred haplotypes. H_C is further split into two subclades (referred to as H_{C1} and H_{C2}). In order to estimate the TMRCA of the three inferred haplotype clades, we applied a phylogeny-based method[44] based on the measure ρ, the average pairwise difference between the two haplotype clusters. The result of ρ was equal to 10.58, so that, with the use of a mutation rate based on 77 fixed differences between chimpanzee and human and a separation time of 6 MY,[45] we estimated a TMRCA of 1.65 MY (SD: 352 KY). In order to obtain a second TMRCA estimate, we used GENETREE, which is based on a maximum-likelihood coalescent analysis.[46,47] The method assumes an infinite-site model without recombination, and, therefore, haplotypes and sites that violate these assumptions need to be removed. In this case, 16 single segregating sites had to be removed. The resulting gene tree, rooted with the use of the chimpanzee sequence, is partitioned into three major branches (Figure S1). A maximum-likelihood θ estimate (θ_{ML}) of 4 was obtained, resulting in an estimated effective population size (N_e) of 10667, a value comparable to most figures reported in the literature.[51] With this method, the TMRCA amounted to 1.05 MY (SD: 293 KY). A third TMRCA estimate of 1.56 MY was obtained by applying a previously described method[52] that calculates the average sequence divergence separating the MRCA and each of the chromosomes. All TMRCAs are not unusually deep; estimates for neutrally evolving autosomal human loci range between 0.8 and 1.5 MY.[51]

The structure of the inferred haplotype genealogy deserves some comments. The allelic status at amino acid

Table 3. Multilocus HKA Results for *ADRB2*

	Multilocus HKA		Multilocus HKA Excluding *ADRB2*	
Pop.	Likelihood[a]	Max Cell Value[b]	Likelihood[a]	Max Cell Value[b]
YRI	16.94 (0.008)	5 (<0.001)	10.66 (0.19)	1.84 (0.16)
EU	28.54 (0.008)	6.52 (0.010)	20.53 (0.07)	2.55 (0.28)
EAS	30.07 (0.019)	10.38 (<0.001)	19.05 (0.17)	3.71 (0.18)

[a] Sum of deviations, p values in parentheses.
[b] Maximum cell value test, p values in parentheses.

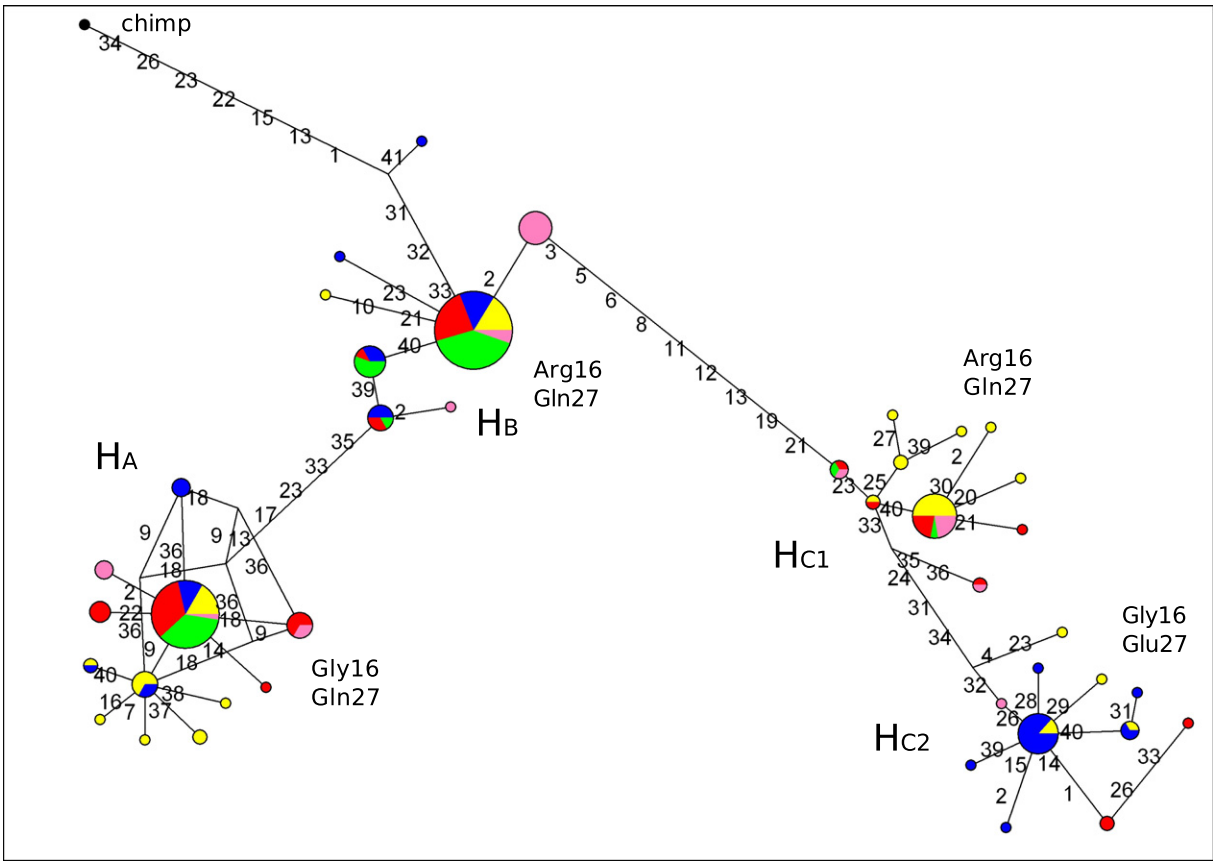

Figure 1. Genealogy of *ADRB2* Inferred Haplotypes Reconstructed through a Median-Joining Network
Each node represents a different inferred haplotype, the size of the circle proportional to frequency. Nucleotide differences between haplotypes are indicated on the branches of the network. Circles are color coded according to population (yellow: YRI, green: NSA, blue: EU, red: EAS, pink: AUA). The chimpanzee sequence is also shown (black circle). Note that the relative position of mutations along a branch is arbitrary. The amino acid status at positions 16 and 27 is shown for the four major inferred haplotypes.

positions 16 and 27 is shown above each haplogroup and indicates that chromosomes carrying Arg16/Gln27 alleles are split among H_B and H_{C1} in all population samples except for EU (there are no EU chromosomes in haplogroup C1); conversely, both Gly16/Gln27 and Gly16/Glu27 chromosomes cluster together to H_A and H_{C2}, respectively. Therefore, we analyzed the location and possible functional significance of variants along the branches separating H_A-H_B, H_B-H_{C1}, and H_{C1}-H_{C2}.

H_A and H_B are separated by five mutations (Figure 1). In addition to codon 16, one of them (rs12654778) falls within a noncoding region, upstream of the 5′ UTR, that is highly conserved among mammals. With respect to variants separating H_B and H_{C1}, all of them are located upstream of the transcription start site, suggesting that the two clades have been maintained by transcription-regulatory variants. Analysis of sequence conservation along this genomic region indicated that variant 13 (rs17778257) is located within an element highly conserved among placental mammals. Further supporting their role as regulatory variants, both of these SNPs located within highly conserved sequences have previously been identified as *cis*-acting expression QTLs in a genome-wide analysis.[53]

Drysdale and colleagues[54] had previously analyzed *ADRB2* haplotype structure: their haplotypes 2 and 4, corresponding to H_B and H_{C1}, showed differential promoter activity, in line with the vision whereby variants along branch H_B/H_{C1} affect gene expression levels. Finally, H_{C1} and H_{C2} differ with respect to codons 16 and 27.

There are different possible reasons underlying selection signatures at human loci. Therefore, we calculated the ratio of observed heterozygosity to expected gene diversity. In order to obtain an estimate of this parameter in the human genome, ratios were also calculated for 5 kb windows deriving from NIEHS genes. Surprisingly, the observed heterozygosity to expected gene diversity for *ADRB2* in EU and EAS falls below the fifth percentile of values obtained from NIEHS genes (Table S3). We therefore wished to verify whether any deviation from Hardy-Weinberg equilibrium (HWE) could be observed. In EU, who lack inferred haplotypes in clade H_{C1}, typing of rs1042714 (Arg16Gly) and rs1042713 (Gln27Glu) allows unambiguous reconstruction of the six possible genotypes deriving from the segregation of the three *ADRB2* inferred haplotypes. Analysis of the 60 unrelated CEPH individuals in HapMap indicated a marginally significant deviation from

Table 4. Genotype Counts, Inferred Haplotype Frequencies, and HWE p Values in Four Populations

	Genotype Counts								Haplotype Frequencies			
Pop.	**Count**	**H_AH_A**	**H_BH_B**	**H_CH_C**	**H_AH_B**	**H_AH_C**	**H_BH_C**	**Sample Size**	**H_A**	**H_B**	**H_C**	**HWE p Value**
CEU[d]												
All	OBS[a]	5	7	19	11	5	13	60	0.22	0.32	0.46	0.0356
	EXP[b]	2.82	6.02	13.07	8.23	12.13	17.73					
Dutch[e]												
All	OBS[a]	315	1376	1776	1257	1481	2986	9191	0.18	0.38	0.44	0.256
	EXP[b]	308.5	1330.9	1749.1	1281.6	1469.3	3051.5					
	RF[c]	0.99	1	0.99	0.96	0.99	0.96					
Females	OBS[a]	175	759	996	700	845	1603	5078	0.19	0.38	0.44	0.094
	EXP[b]	177.6	717.9	969.7	714.1	829.9	1668.8					
	RF[c]	0.93	1	0.97	0.93	0.96	0.91					
Males	OBS[a]	140	608	780	557	637	1383	4105	0.18	0.38	0.44	0.874
	EXP[b]	133.0	605.3	780.3	567.5	644.3	1374.6					
	RF[c]	1	0.95	0.95	0.93	0.94	0.96					
French[f]												
All	OBS[a]	58	157	181	158	190	385	1129	0.205	0.380	0.415	0.053
	EXP[b]	47.67	162.63	194.41	176.11	192.55	355.63					
	RF[c]	1	0.79	0.77	0.74	0.81	0.89					
Females	OBS[a]	30	85	89	63	98	197	562	0.20	0.38	0.42	0.009
	EXP[b]	21.8	82.4	99.6	84.8	93.2	181.2					
	RF[c]	1	0.75	0.65	0.54	0.76	0.79					
Males	OBS[a]	28	72	92	95	92	188	567	0.21	0.38	0.41	0.421
	EXP[b]	26.0	80.6	94.8	91.5	99.3	174.9					
	RF[c]	1	0.83	0.90	0.96	0.86	1					
Italian[g]												
All	OBS[a]	35	109	78	132	135	165	654	0.26	0.39	0.35	0.110
	EXP[b]	43.4	101.4	79.5	132.7	117.5	179.5					
	RF[c]	0.70	0.94	0.85	0.87	1	0.83					
Females	OBS[a]	13	53	40	63	69	72	310	0.25	0.39	0.36	0.0368
	EXP[b]	20.1	46.8	39.4	61.4	56.3	85.9					
	RF[c]	0.53	0.92	0.83	0.84	1	0.68					
Males	OBS[a]	22	56	38	69	66	93	344	0.26	0.40	0.34	0.876
	EXP[b]	23.3	54.6	40.1	71.3	61.1	93.6					
	RF[c]	0.88	0.95	0.88	0.90	1	0.92					

[a] Observed count.
[b] Expected count.
[c] Relative fitness.
[d] CEPH samples extracted from the HapMap Project.
[e] Dutch samples derived from Sethi et al.[55]
[f] French samples derived from Dallongeville et al.[56]
[g] Italian samples derived from this study.

HWE and an excess of homozygotes (Table 4). Therefore, we searched the literature, looking for *ADRB2* genotypes at both polymorphisms in large European cohorts. In the two available studies,[55,56] we observed a marginally significant deviation in women in one study only, which we replicated in our own genotyped Italian sample (Table 4).

Table 5. Composite Likelihood Ratio Test for *ADRB3*

	Test 1[a]		Test 2[b]		Test 3[c]
Pop.	LR[d]	GOF[e]	LR[d]	GOF[e]	LR[d]
YRI	12.74 (0.002)	453 (0.38)	12.45 (<0.0001)	3438 (0.060)	7.26 (0.60)
EU	9.34 (0.002)	550 (0.040)	3.25 (0.07)	1615 (0.044)	n.a.[f]

[a] Distinguishing ancestral and derived allele.
[b] Nondistinguishing ancestral and derived allele.
[c] Distinguishing ancestral and derived allele only for inferred haplotypes carrying the putative selected allele (W64).
[d] Likelihood ratio, p values in parentheses.
[e] Goodness-of-fit test, p values in parentheses.
[f] Not analyzed.

Because deviations from HWE are not observed in all cohorts and display marginally significant p values (with the exception of the French female sample), we cannot reach definitive conclusions, although the possibility that the results are due to genotyping artifacts or other unrecognized biases (e.g., a deletion segregating in the populations) is unlikely, given that (1) HWE deviation was observed in women but not in men, (2) different studies used different genotyping techniques, and (3) by analyzing 73 Italian trios, we did not detected inconsistent segregation of *ADRB2* inferred haplotypes (not shown).

A Selective Sweep at *ADRB3*

As reported above, negative values of frequency-spectrum-based statistics were obtained for *ADRB3*. This result can be interpreted as evidence of either purifying selection or selective sweep, given that both processes result in an excess of low-frequency variants. Fay and Wu's H is usually applied to distinguish between the two possibilities.[38] Negative H values indicate an excess of high-frequency derived alleles, a finding consistent with the action of directional but not purifying selection. Given that selective sweeps are expected to affect genomic regions surrounding the selected variant(s), we calculated H (and performed all successive analyses) over the whole resequenced region (which extends ~3.8 kb downstream of the 3′ UTR). A significantly negative H value was obtained for YRI and EU ($H = -6.72$, $p = 0.014$ and $H = -7.33$, $p = 0.045$, respectively).

In order to obtain a further confirmation, we applied a composite-likelihood ratio test (CLR[57]), which evaluates the local reduction of variation and skew of the frequency spectrum. At first, we evaluated statistical significance of likelihood ratio (LR) values in two ways: distinguishing ancestral from derived alleles and then not distinguishing allele states (Test 1 and Test 2, respectively). Results are summarized in Table 5 and indicate that, after coalescent simulations, data for Africans fit significantly better a hitchhiking event for both conditions; conversely, for Europeans, only Test 1 gave significant results. Given that CLR is not robust to demographic history, we applied a goodness of fit (GOF) test;[58] this method specifically tests how well a selective sweep model fits the data, as opposed to a generalized alternative model, by simulating genealogies under directional selection. Thus, nonsignificant p values represent a good fit of the sweep model to the data, whereas low p values fall within the range of effects that can also be generated by demographic events such as population bottlenecks or undetected population structure. For YRI, the GOF p values suggest that rejection of neutrality by the CLR test is more likely due to a selective sweep than to demographic history (Table 5). In contrast, the GOF test fails to discard a relevant demographic effect for Europeans.

As reported in the Introduction, the ancestral R64 allele has been associated with obesity and insulin resistance in some populations, and its frequency is relatively low in Europeans, Asians, and Africans.[29] As a consequence, the W64 derived allele, the only high-frequency derived allele in the *ADRB3* coding sequence, might be a good candidate to represent the selected variant. Therefore, we performed an additional test for YRI under the assumption that an incomplete sweep has been ongoing, with the W64 allele being the selected site. To this aim, only inferred haplotypes carrying the W64 allele were selected and LR value was compared with those obtained by simulating data under a complete sweep model[59] (Test 3). Nonsignificant p values (Table 5) make this evolutionary hypothesis very appealing.

Haplotype analysis indicated that a total of 27 *ADRB3* inferred haplotypes can be identified (Figure 2), but one inferred haplotype carrying the W64 allele occurred 55 times and accounted for 45.8% and 71% of African and European chromosomes, respectively. The Hudson haplotype test[60] for the W64 variant indicated that significantly fewer ($p = 0.0046$) inferred haplotypes than expected under neutrality are observed in YRI. We then constructed a median-joining network for *ADRB3* inferred haplotypes (Figure 3). Given the presence of a few recombination events immediately downstream of the transcription end site, the gene region extending from the most 5′ resequenced nucleotide to the 3′ UTR end was used for this analysis. As shown in Figure 3, all haplotypes carrying the W64 allele (mutation 11 in the network) cluster together or are few steps away from the major haplotype, except for a divergent branch of African haplotypes.

These data are consistent with directional selection at the *ADRB3* locus in YRI. With respect to the European sample, most tests failed to reject neutrality; yet, it should be noted that only eight SNPs are segregating in this population, possibly resulting in insufficient power to detect deviations from the neutral expectations.

Discussion

In this work, we wished to analyze the evolutionary history of *ADRB* genes in humans, with particular concern to selective patterns. Indeed, it has been proposed[61,62] that population genetics approaches can be regarded as a counterpart to

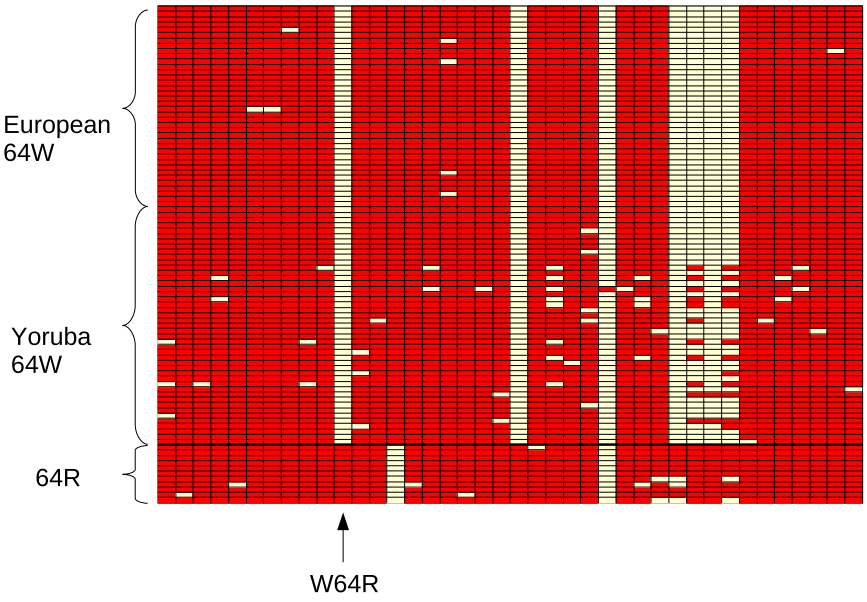

Figure 2. Analysis of *ADRB3* Inferred Haplotypes
Polymorphic positions are color coded according to their allelic state (white: derived, red: ancestral). The position of the W64R variant is shown, and inferred haplotypes are ordered on the basis of the allele at codon 64.

classic association studies, in that by focusing on the effect of susceptibility alleles on evolutionary fitness, they allow inference of functional relevance. Consistently, it has been shown[63] that genes targeted by selection acting on segregating variants are more likely to be associated with human diseases.

Data reported herein indicated that the genomic region covering the promoter and coding sequences of *ADRB2* displays high nucleotide diversity, an excess of intermediate-frequency alleles, and a higher level of within-species diversity in comparison to interspecific divergence. All of these features strongly suggest the action of balancing selection. In particular, analysis of inferred haplotypes indicated that the three clades are likely to be maintained by both coding and regulatory balanced SNPs, being consistent with a model of multiallelic balancing selection. As shown by the median-joining network (Figure 1), three lineages are observed (as opposed to two expected clades in the case of a biallelic model). This is in line with values of the test statistics (Tajima's D and Fu and Li's F* and D*), which are not strikingly positive; the skew toward intermediate frequency variants tends to be less marked in a multiallelic selection model than in the case of biallelic selection. Estimation of TMRCAs for *ADRB2* inferred haplotype clades indicated that they are not unusually deep. Thus, an alternative possibility is that variants in the gene are going through a selective sweep; indeed, both balancing and positive selection are initiated by the spread in a population of a newly selected allele(s) until either selection opposes (balanced situation) or promotes (complete sweep) its fixation. Population genetics analyses such as the one reported herein provide a snapshot of a dynamic evolutionary process, making it difficult to precisely determine the underlying selective regime.

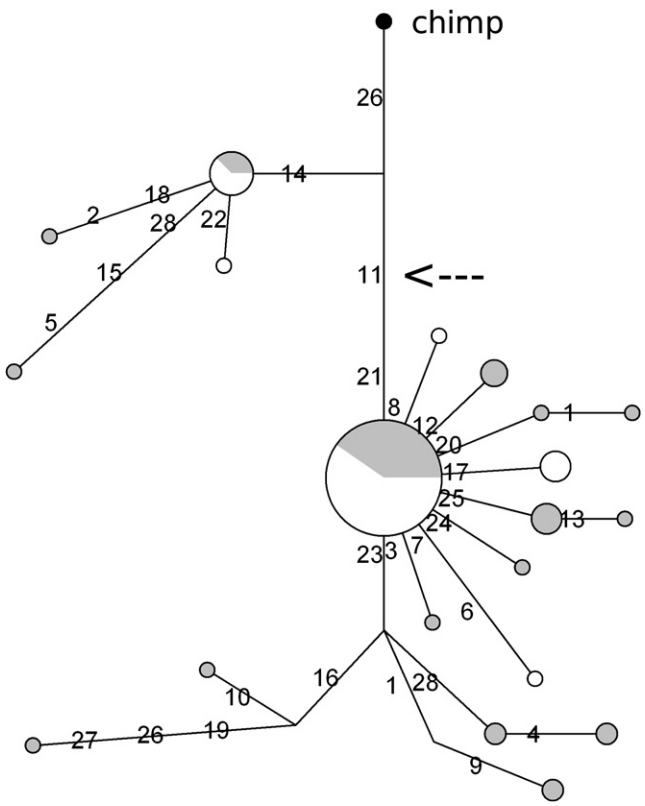

Figure 3. Genealogy of *ADRB3* Inferred Haplotypes Reconstructed through a Median-Joining Network
Each node represents a different inferred haplotype, the size of the circle proportional to frequency. Nucleotide differences between haplotypes are indicated on the branches of the network. Circles are color-coded according to population (gray: YRI, white: EU). The chimpanzee sequence is also shown (black circle), and the arrow indicates the W64 polymorphism.

It is worth mentioning that the *ADRB2* inferred haplotype structure might harbor implications for association studies. Indeed, whereas in Europeans, all chromosomes carrying alleles Arg16 and Gln27 display the same promoter structure (clade H_B), in all other population samples, inferred haplotypes harboring these coding variants are split into two groups (H_B and H_{C1}) with different alleles in the promoter region and, possibly, different transcriptional activity.[53,54] This observation might partially explain the low consistency among association studies in different population samples.

In analogy to the underlying selective regimes, the nature of selective pressures acting on human genes is often

difficult to identify. Our analyses of different European cohorts suggests that *ADRB2* inferred haplotypes deviate from HWE in females but not in males. Deviations from HWE in a sex-dependent manner have previously been reported for variants in the *BRCA2* gene (MIM 600185) in humans.[64] Still, in the case of *ADRB2*, deviations from HWE often display marginally significant p values, and genotypes with maximum and minimum relative fitness are different across the three European populations (Table 4), an observation difficult to reconcile with simple selection models. Previous theoretical modeling has demonstrated that different situations, including maternal (parental) selection[65,66] and epistasis,[67] can result in complex genotype distributions and oscillations in genotype frequencies. In theory, both effects might apply to *ADRB2*, although maternal selection is supported by previous findings indicating that both fetal[18,22] and maternal[19–21] genotype at single *ADRB2* SNPs predispose to preterm delivery and cerebral palsy. Unfortunately, none of these studies addressed the role of sex in pregnancy outcome. A known example of genetic variation influencing intrauterine viability concerns the methylenetetrahydrofolate reductase gene (*MTHFR* [MIM 607093]),[68] and, in analogy to what we observed for *ADRB2*, deviations from HWE in different human populations have been observed, as an excess of either homozygotes or heterozygotes in Italy and Finland, respectively.[69] Another instance involves the phosphoglucomutase 1 (*PGM1* [MIM 171900]) gene. The joint maternal-neonatal distribution of *PGM1* genotypes has been shown to deviate from that expected under HWE,[70] and the same authors reported that a significant association is observed between birth weight and mother-newborn *PGM1* genotype in infant females but not males.[71]

Additional studies will be required for verifying whether selection for intrauterine viability (or viability in adult life) does occur on *ADRB2* haplotypes and determining whether it is responsible for the selection signature that we observed. Indeed, the central role played by *ADRB2* in many physiologic pathways suggests that it might have been a target of diverse selective pressures.

Polymorphic variations in the gene have been associated with three extremely frequent conditions: asthma, hypertension, and obesity (or insulin resistance). Some recent theories[29,30] proposed these common diseases to be widespread in human populations because susceptibility alleles have been selected for during human evolution and have more recently become unfavorable as a consequence of changed environmental condition and lifestyle. In particular, it has been suggested[29] that alleles responsible for type 2 diabetes might have evolved as "thrifty" variants in ancient populations; similarly, variants predisposing to asthma and atopy possibly conferred increased resistance to parasites in endemic areas. Finally, susceptibility alleles for hypertension were proposed to have conferred selective advantage to populations adapted to hot and humid climates in order to maximize sodium retention or vascular reactivity.[29,30] These evolutionary-framework hypotheses might well fit *ADRB2* coding variants. Indeed, the ancestral Glu27 allele has been associated with obesity in a number of studies and the three β-adrenergic receptors are known to play pivotal roles in regulating metabolic rate and thermogenesis.[72] Also, the ancestral Gly16 variant has been associated with both asthma and resistance to *A. lumbricoides* infection,[23] and a latitudinal cline has been identified for Gly16 and Glu27, the two ancestral variants being possibly adapted to hot climates.[73] The ancestral amino acid state at codons 16 and 27 might therefore have conferred some selective advantage with respect to energy storage, ascariasis, and vascular reactivity. Yet, some recent evidence suggests that additional selective pressures might have been acting on *ADBR2* variants. Indeed, signaling via *ADRB2* and heterotrimeric guanine nucleotide-binding proteins has been shown to regulate the entry[74,75] and intracellular maturation[75] of *Plasmodium falciparum* in erythrocytes. Malaria (MIM 611162) is thought to have exerted a strong selective pressure on humans, resulting in the selection of hundreds of genetic variants conferring some disease protection; it is therefore conceivable that *Plasmodium* infection has exerted some selective pressure on *ADRB2* polymorphisms, as well.

In analogy to *ADRB2* the ancestral susceptibility model might apply to *ADRB3*; other authors[29] suggested that the *ADRB3* Arg64 allele might represent a thrifty variant. Although the contribution of *ADRB3* genotypes to the etiology of obesity and type 2 diabetes is still controversial, a meta-analysis[76] indicated that the Arg64 variant plays a modest but significant role in the susceptibility to non-insulin-dependent diabetes mellitus. Moreover, a consistent association with younger age at onset of diabetes has been reported in several analyses.[76–79] Similarly, a study[80] based on paired sibling analysis indicated a significant association between the *ADRB3* Arg64 allele and obesity. In line with these findings, homozygotes for the Arg64 allele display lower metabolic resting rates as compared to those of subjects carrying at least one Trp64 variant,[81] and the Arg64 allele might associate with mild gestational diabetes and increased weight gain in pregnancy.[82] Despite its appeal, the thrifty-genotype hypothesis has received limited experimental support, and a limited number of studies[83–85] have attempted to apply population genetics approaches to detect selection signatures at thrifty variants. Here, we have shown that the the derived Trp64 variant, which possibly represents a nonthrifty allele, has raised in frequency in human populations as a result of directional selection.

The specific selective pressure underlying this event remains to be identified, although changes in diet, which are thought to have occurred during recent human history, might have resulted in widespread selection of genes involved in energy metabolism.[86]

Supplemental Data

Supplemental Data include one figure and three tables and can be found with this article online at http://www.ajhg.org/.

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Web Resources

The URLs for data presented herein are as follows:

MaxDip, http://genapps.uchicago.edu
NIEHS Environmental Genome Project, http://egp.gs.washington.edu
Online Mendelian Inheritance in Man, www.ncbi.nlm.nih.gov/Omim/
R Project, www.r-project.org
Seattle SNPs, http://pga.mbt.washington.edu

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