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Genome-wide linkage scan for submaximal exercise heart rate in the HERITAGE family study

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Spielmann N, Leon AS, Rao DC, Rice T, Skinner JS, Rankinen T, Bouchard C. Genome-wide linkage scan for submaximal exercise heart rate in the HERITAGE family study. *Am J Physiol Heart Circ Physiol* 293: H3366–H3371, 2007. First published October 5, 2007; doi:10.1152/ajpheart.00042.2007.—The purpose of this study was to identify regions of the human genome linked to submaximal exercise heart rates in the sedentary state and in response to a standardized 20-wk endurance training program in blacks and whites of the HERITAGE Family Study. A total of 701 polymorphic markers covering the 22 autosomes were used in the genome-wide linkage scan, with 328 sibling pairs from 99 white nuclear families and 102 pairs from 115 black family units. Steady-state heart rates were measured at the relative intensity of 60% maximal oxygen uptake (HR60) and at the absolute intensity of 50 W (HR50). Baseline phenotypes were adjusted for age, sex, and baseline body mass index (BMI) and training responses (posttraining minus baseline, Δ) were adjusted for age, sex, baseline BMI, and baseline value of the phenotype. Two analytic strategies were used, a multipoint variance components and a regression-based multipoint linkage analysis. In whites, promising linkages (LOD $>$ 1.75) were identified on 18q21-q22 for baseline HR50 (LOD = 2.64; P = 0.0002) and ΔHR60 (LOD = 2.10; P = 0.0009) and on chromosome 2q33.3 for ΔHR50 (LOD = 2.13; P = 0.0009). In blacks, evidence of promising linkage for baseline HR50 was detected with several markers within the chromosomal region 10q24-q25.3 (peak LOD = 2.43, P = 0.0004 with D10S597). The most promising regions for fine mapping in the HERITAGE Family Study were found on 2q33 for HR50 training response in whites, on 10q25-26 for baseline HR60 in blacks, and on 18q21–22 for both baseline HR50 and ΔHR60 in whites.

exercise training; linkage; quantitative trait loci; genotype

IN RECENT YEARS, a large body of evidence has clearly established sedentarism as a risk factor for a number of diseases that become more prevalent with age in both sexes. In contrast, physical activity regularly performed in a variety of settings is considered beneficial because of favorable metabolic and cardiovascular outcomes (9, 17, 30, 31, 37, 49). Previous studies have shown associations between elevated heart rate (HR) at rest and increased risk of both all-cause and cardiovascular mortality (7, 18, 21, 22, 25, 26, 33). Although HR during submaximal exercise is known to decrease in response to regular endurance training (16, 51), its recovery after maximal and submaximal exercise is considered a powerful predictor of mortality (14, 15). However, interindividual differences in training-induced changes in HR are considerable.

In the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study, the steady-state HR at the absolute intensity of 50 W (HR50) and relative intensity of 60% (HR60) of maximal oxygen uptake ($\dot{V}O_{2max}$) were significantly reduced after 20 wk of endurance training (51). The maximal heritability estimates for baseline HR50 and HR60 reached 59 and 46%, respectively. The maximal heritabilities were 34 and 29% for ΔHR50 and ΔHR60, respectively, for the changes in response to a 20-wk endurance training program (Δ, posttraining minus baseline) (5). Furthermore, complex segregation analysis supported the hypothesis of a major recessive gene effect on baseline HR50 and a major dominant gene effect on ΔHR50 in the same families (4).

The identification of the genes and mutations affecting exercise HR would lead to a better understanding of the biology of adaptation to exercise. The objective of this study was to perform a genome-wide linkage scan for HR50 and HR60 measured in the sedentary state and in response to a 20-wk endurance training program in the HERITAGE Family Study.

MATERIALS AND METHODS

Subjects. The HERITAGE Family Study design, inclusion criteria, and protocol have been described previously (8). A total of 503 white subjects (245 men and 258 women) from 99 nuclear families and 276 black subjects (91 men and 185 women) from 115 family units were included. Complete training response data were available for 472 whites (229 men and 243 women) and 254 blacks (88 men and 166 women). The maximum number of sibling pairs available was 328 and 102 in whites and blacks, respectively. All subjects were healthy and sedentary at baseline. Sedentary was defined as no regular physical activity over the previous 6 mo. The study protocol was approved by the Institutional Review Boards at each of the five participating centers of the HERITAGE Family Study consortium. Written informed consent was obtained from each participant.

Exercise training program. Subjects completed a 20-wk endurance training program (3 days per week for a total of 60 exercise sessions) under supervision using Universal Aerobicycles (Cedar Rapids, IA), which were monitored electronically by the Fit Net system to maintain the participants' HR at levels associated with fixed percentages of their $\dot{V}O_{2max}$. The training program started at 55% of $\dot{V}O_{2max}$ for 30 min per session and gradually increased to 75% of $\dot{V}O_{2max}$ for 50 min per session during the last 6 wk of training. All training sessions were supervised on site, and adherence to the protocol was strictly monitored.

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Submaximal exercise test. Before and after the 20-wk training program, each subject completed two submaximal exercise tests on separate days. Submaximal exercise tests at 50 W and at 60% of $\dot{V}O_{2max}$ were conducted on a cycle ergometer. Subjects exercised 8–12 min at an absolute work load of 50 W and at a relative power output equivalent to 60% of $\dot{V}O_{2max}$, with a 4-min period of seated rest between the exercise periods. HR was monitored throughout the test with an electrocardiogram, and two HR values were recorded once steady state had been achieved. The HR values presented in this article represent the mean of two submaximal tests (i.e., 4 individual measurements), both before and after training. A detailed description of the exercise test methodology has been reported previously (46). The reproducibility of the submaximal exercise HR measurements was very high, with coefficients of variation and intraclass correlations ranging from 4.4 to 5.1% and 0.88 to 0.89, respectively (52).

Data adjustment. Baseline HR50 and HR60 measurements were adjusted for the effects of sex, age, and body mass index (BMI) using stepwise multiple regressions (42). Training response phenotypes were additionally adjusted for baseline value of the phenotype. In summary, HR50 and HR60 phenotypes were regressed on up to a third-degree polynomial in age and on BMI (separately within race-by-gender-by-generation subgroups). Only significant terms (5% level) were retained (i.e., the model did not need to be saturated). The residuals from this regression were then standardized to zero mean and unit variance and constituted the analysis variables.

Molecular studies. A total of 701 markers covering the 22 autosomes with a mean spacing of 4.1 Mb on the physical map were used. PCR conditions and genotyping methods have been reported previously (12). DNA sequencers from LI-COR were used to detect the PCR products, and genotypes were scored semiautomatically using the software SAGA. Incompatibilities of Mendelian inheritance were checked, and markers showing incompatibilities were regenotyped (<10% were retyped). Microsatellite markers were selected mainly from the Marshfield panel version 8a. In addition to this panel of markers, some restriction fragment length polymorphism (RFLP) markers were integrated in the scan. These RFLPs included candidate genes relevant for HERITAGE phenotypes such as submaximal HR. Map locations of the markers were taken from the Build 35 of the National Center for Biotechnology Information (NCBI) physical map.

A quantitative trait loci (QTL) of interest on chromosome 10q25 included two potential candidate genes, the adrenergic receptors α_{2A} (ADRA2A) and β_1 (ADRB1), which were further investigated by genotyping 16 linkage disequilibrium bin tagging single-nucleotide-polymorphisms (tagSNP) from the SeattleSNP database (http://pga.mbt.washington.edu/; see Supplemental Tables 1 and 2 for details). (Supplemental data for this article is available at the *American Journal of Physiology-Heart and Circulatory Physiology* website.) SNP genotyping was performed using a primer extension method with fluorescence polarization detection (FP-TDI). Changes in fluorescence polarization after excitation of the samples by plane-polarized light were measured using a Victor2 plate reader (PerkinElmer Life Sciences). The allele calling was done using the SNPscorer genotyping software (PerkinElmer Life Sciences).

Statistical analyses. Linkage analysis was performed using multipoint variance components and regression-based models as implemented in MERLIN (2). Under the variance components model, a phenotype is influenced by the additive effects of a trait locus (g), a residual familial background modeled as a pseudopolygenic component (GR), and a residual nonfamilial component (r). The effects of the trait locus and the pseudopolygenic component on the phenotype represent the locus-specific (h^2g) and residual genetic (h^2r) heritabilities. The linkage hypothesis is tested by restricting the trait locus heritability to zero. A likelihood ratio test contrasts the null hypothesis ($h^2g = 0$) with the alternative (h^2g estimated). The difference in -2 ln L (minus twice the log likelihood) between the null and alternate hypotheses is asymptotically distributed as a 50:50 mixture of a χ_1^2 and a point mass at zero, and the *P* value is one-half of that associated with the χ^2 value with 1 df (difference in the number of parameters estimated) (2). All analyses were conducted separately in blacks and whites. Promising linkage was defined as logarithm of the odds (LOD) >1.75 ($P < 0.0023$) as recommended by Rao and Province (41).

As a follow-up of the linkage signal observed on chromosome 10q25 in blacks, association studies were performed using 6 and 10 tagSNPs on ADRA2A and ADRB1 loci, respectively. Associations were explored using two complementary methods: a variance components and likelihood ratio test-based procedure in the QTDT software package and a MIXED model-based procedure in the SAS software package. The total association model of the QTDT software utilizes a variance-components framework to combine a phenotypic means model and the estimates of additive genetic, residual genetic, and residual environmental variances from a variance-covariance matrix into a single-likelihood model (1). The evidence of association is evaluated by maximizing the likelihoods under two conditions: the null hypothesis (L_0) restricts the additive genetic effect of the marker locus to zero ($\beta_a = 0$), whereas the alternative hypothesis does not impose any restrictions to β_a. The quantity of twice the difference of the log likelihoods between the null and the alternative hypotheses, $2[\ln(L_1) - \ln(L_0)]$, is distributed as χ^2 with 1 df. A dominance effect can be tested in a similar manner, but the alternative hypothesis model includes estimates for both additive (β_a) and dominant ($\beta_a \times \beta_a$) genetic effects, and the likelihood ratio test is based on χ^2 distribution with 2 df (1). In the MIXED model, nonindependence among family members was adjusted for by using a sandwich estimator, which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the SEs and, consequently, hypothesis tests are adjusted for the within-family dependencies. The method is general, assuming the same degree of dependency among all members within a family. The pairwise linkage disequilibrium (LD) between the SNPs was assessed using the ldmax program available in the GOLD software package (1).

RESULTS

The descriptive characteristics of 503 white and 276 black subjects are presented in Table 1. Pre- and posttraining data for HR50 and HR60 in the HERITAGE Family Study have been described and discussed elsewhere (51).

In whites, the strongest evidence of linkage for baseline HR50 was detected on chromosome 18q21.33 (LOD = 2.64, $P = 0.0002$) (Fig. 1). In addition, promising linkage for ΔHR50 was detected on chromosome 2q33.3 (LOD = 2.13, $P = 0.0009$) and for ΔHR60 on chromosome 18q21.1 (LOD = 2.10, $P = 0.0009$) (Table 2). In blacks, five markers within the chromosomal region 10q24–q25.3 showed evidence of linkage

Table 1. *Descriptive characteristics of white and black subjects of the HERITAGE Family Study*

Variables	Black Subjects		White Subjects	
	Mean	SD	Mean	SD
Age, yr	32.9	11.6	35.4	14.5
BMI, kg/m^2	28.0	6.2	25.9	5.0
Baseline	*n* = 276		*n* = 503	
HR50, beats/min	125.7	19.0	117.4	17.3
HR60, beats/min	135.5	15.6	141.4	17.1
Training response	*n* = 254		*n* = 472	
HR50, beats/min	−12.1	10.6	−11.0	9.9
HR60, beats/min	−2.2	10.6	−5.4	10.4

A total of 503 white and 276 black subjects were included in the study (baseline); complete training response data were available for 472 white and 254 black ssubjects. BMI, body mass index; HR50, heart rate at 50 W; HR60, heart rate at 60% of maximal oxygen uptake.

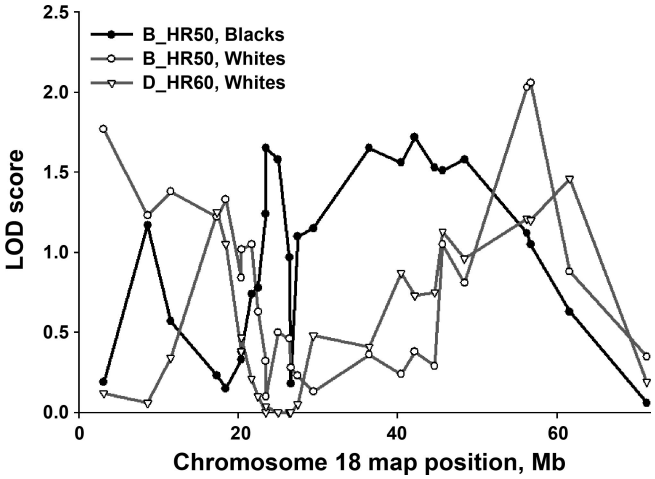

Fig. 1. Linkage (LOD) results on chromosome 18 for baseline heart rate at 50 W (B_HR50) in black and white subjects and heart rate at 60% of maximal oxygen uptake ($V_{O_{2max}}$) training response (D_HR60) in white subjects. LOD, logarithm of the odds.

for baseline HR60 with peak linkage detected with marker D10S597 (LOD = 2.43, P = 0.0004). Further evidence of linkage with baseline HR50 was found on chromosomes 3q11.1–12.3 (LOD = 1.88, P = 0.002), 9q32–33.1 (LOD = 1.93, P = 0.001), and 10q25.1 (LOD = 1.84, P = 0.002) (Table 3).

The QTLs for baseline HR50 and HR60 on chromosome 10q25 detected in blacks (Fig. 2) harbor two excellent candidate genes for exercise HR: adrenergic receptors β_1 (ADRB1) and α_{2A} (ADRA2A). We performed association tests using 6 ADRA2A and 10 ADRB1 SNPs in black subjects. The Arg389Gly (rs1801253) variant in the ADRB1 locus was associated with baseline HR50 in siblings (P = 0.02 MIXED model; P = 0.03 QTDT dominance model) and in the whole cohort (P = 0.04 MIXED model). No significant association was found between the Arg389Gly (rs1801253) and baseline HR60.

The linkage model of the QTDT captured the same linkage signal with the ADRA2A and ADRB1 tagSNPs for baseline HR50 as did the original genome-wide linkage scan. When the association parameter was added to the model, the signal at the ADRA2A locus remained virtually unchanged. However, at the ADRB1 locus, the signal weakened from LOD = 2.07 to LOD = 0.62 at Arg389Gly (rs1801253), suggesting that the SNP may explain a substantial portion of the QTL variance (Fig. 3).

DISCUSSION

A genome-wide search for QTLs influencing submaximal exercise HR50 and HR60 before and in response to a 20-wk endurance training program was undertaken in the HERITAGE Family Study. This is the first study attempting to localize genomic regions affecting submaximal exercise HR. Identifying QTLs and resolving them in terms of genes and mutations would benefit our understanding of basic human exercise physiology.

We identified QTLs on chromosome 18q21–22 for baseline HR50 and HR60 training responses and on chromosome 2q33 for training-induced changes in HR50 in whites. Furthermore, we detected promising linkage signals within the chromosomal region 10q24–q25.1 for baseline HR50 and HR60 in the black HERITAGE Family Study cohort. So far, no other genome-wide linkage scans are available for submaximal exercise HR phenotypes that could be used to compare our results. However, the HyperGen Study reported a QTL for resting HR on chromosome 10q24–q25 near the QTL for sedentary state submaximal exercise HR found in black HERITAGE families (50).

The regulation of the cardiovascular system is under tight control to establish and maintain homeostasis. G protein-coupled receptor signaling is an important component of this regulation. Adrenergic receptors (ARs) are a member of the superfamily G protein-coupled receptors, which bind epinephrine and norepinephrine, thereby mediating their intracellular effects (20). The activation of α_{2A}-ARs in the brain stem leads to a reduction in sympathetic tone, with a resultant decrease in HR and blood pressure (3, 27). α_{2A}-ARs are the principle regulators of catecholamine release, and their absence results in altered sympathetic regulation due to loss of α_{2A}-mediated inhibition of *1*) sympathetic tone in the brain stem and *2*) catecholamine release from the sympathetic nerve terminus (3). β_1-ARs are key mediators of the sympathetic inotropic and chronotropic effects in the heart response to catecholamines (10, 36). Furthermore, β_1-ARs play a dominant role in adrenergic-mediated increases in HR by regulating contractility, and their absence results in a decrease in the average HR (19). A nonsynonymous polymorphism, Arg389Gly, has been detected in the coding region of the β_1-AR gene (48). In vitro, the wild-type Arg389 form produces increased high-affinity agonist binding and enhanced adenylyl cyclase activities compared with the 389Gly form (32, 34, 47). In addition, the Arg389 variant has been reported to have greater inotropic and cAMP responses to norepinephrine than the 389Gly variant (44), but others have not confirmed these genotype-dependent differences (35, 45). Although higher resting HR has been reported

Table 2. *Promising linkages with submaximal HR at baseline and in response to training in whites*

				Variance Components		Regression Analysis	
Marker	Chromosome	Trait	Map Position, Mb	LOD Score	P Value	LOD Score	P Value
Baseline							
D18S38	18q21.32	HR50	56.697	2.06	0.001	2.64	0.0002
Training response							
D2S154	2q33.3	HR50	208.510	1.94	0.001	2.13	0.0009
D18S878	18q22.1	HR60	61.551	1.46	0.005	2.10	0.0009

Promising linkage was defined as a logarithm of the odds (LOD) >1.75.

Table 3. *Linkages for baseline submaximal HR phenotypes in blacks*

Marker	Chromosome	Trait	Map Position, Mb	Variance Components		Regression Analysis	
				LOD Score	*P* Value	LOD Score	*P* Value
D3S2459	3q13.11	HR50	103.658	1.70	0.003	1.88	0.002
D9S154	9q33.1	HR50	118.381	1.93	0.0014	1.66	0.003
D10S603	10q24.2	HR50	102.047	1.53	0.004	1.59	0.003
D10S597	10q25.1	HR50	111.221	1.63	0.003	1.84	0.002
D18S1111	18q21.1	HR50	42.130	1.72	0.002	1.66	0.003
D10S597	10q25.1	HR60	111.221	2.14	0.0009	2.43	0.0004
ADRA2A *Msp*I*	10q25.2	HR60	112.826	2.02	0.001	2.25	0.0006
ADRB1G49S	10q25.3	HR60	115.794	1.88	0.002	1.96	0.001
ADRB1R389G	10q25.3	HR60	115.795	1.88	0.002	1.96	0.001
D10S468	10q25.3	HR60	117.195	1.80	0.002	1.71	0.003

*ADRA2A *Msp*I corresponds to SNP rs1800544.

in siblings homozygous for the Arg389 allele (6, 24), HR response to acute exercise has not been associated with the Arg389Gly genotype (11, 29, 36, 38, 53). In the present study, we found a modest association between the Arg389Gly variant and baseline HR50 in blacks but not in whites. Subjects homozygous for the 389Gly allele showed higher baseline HR50 values compared with the other genotypes. As much as 2.3% of the variance in baseline HR50 was explained by Arg389Gly in black subjects. The implications of the ADRB1 gene SNPs for the HR response to exercise need to be further investigated. It also should be noted that the 1-LOD linkage region on chromosome 10q25 spans almost 20 Mb and contains 118 genes (Supplemental Table 3). The possibility that other genes in the region (in addition to ADRB1) also may affect submaximal exercise HR phenotypes remains to be explored in fine mapping studies.

In addition, we found evidence of linkage on chromosome 18 for several submaximal HR phenotypes in blacks and whites. These results suggest that the general region of chromosome 18q21–q22 may harbor genes that affect submaximal exercise HR in both blacks and whites. The HR50 and ΔHR60 QTL on 18q21.1 coincides with previously reported QTLs for changes in fat mass and percent body fat with training (13) and for submaximal exercise diastolic blood pressure at 50 W in blacks (40).

We also found evidence of promising linkage on chromosome 2q33 for ΔHR50 in whites. This QTL is localized in the vicinity of the HERITAGE QTLs for submaximal exercise cardiac output and stroke volume training responses (2q32.1) (39) and for baseline abdominal visceral fat level (2q36) (43). Furthermore, a gene causing familial primary pulmonary hypertension (BMPR2) is located on this area (28), and our ΔHR50 QTL overlaps with QTLs for resting systolic and diastolic blood pressures reported in Old Order Amish (23).

Our analyses dealt with two HR phenotypes measured during submaximal exercise. Although the phenotypes are correlated, the relationship is not as strong as one might expect. The correlations between adjusted baseline HR50 and HR60 were $r = 0.66$ in whites and $r = 0.57$ in blacks, whereas the correlations between training responses were $r = 0.54$ in whites and $r = 0.49$ in blacks. As these correlations already indicate, HR50 and HR60 are also physiologically different phenotypes. HR50 is defined using the same absolute workload (50 W) for all subjects both before and after training, whereas HR60 is based on workload relative to each individual maxi-

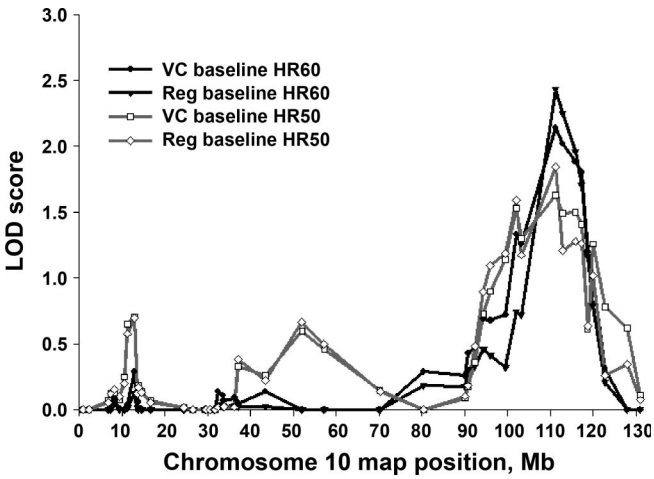

Fig. 2. Linkage results for baseline heart rate at 50 W (HR50) and 60% $\dot{V}O_{2max}$ (HR60) on chromosome 10 in black subjects. VC, multipoint variance-components linkage analysis; Reg, regression-based multipoint linkage analysis.

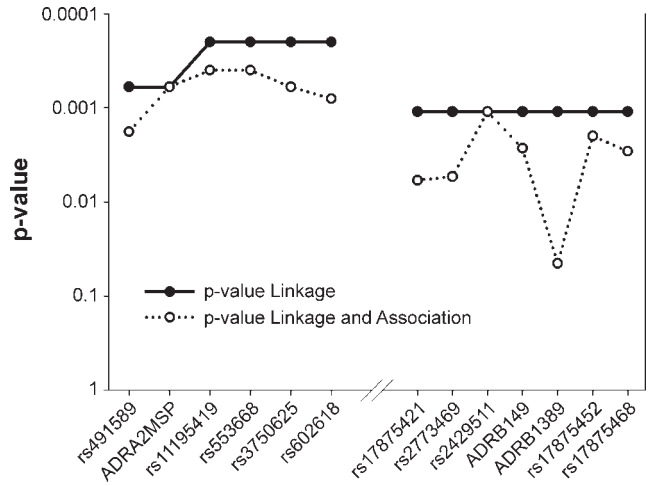

Fig. 3. Results of the QTDT software linkage model with and without an association term for baseline HR50. Evidence of linkage with Arg389Gly in the ADRB1 (adrenergic receptor β_1) gene considerably weakened when the association term is included in the model, suggesting that Arg389Gly partially explains the linkage with baseline HR50. ADRA2, adrenergic receptor α_{2A}.

mal exercise capacity (60% of $\dot{V}O_{2max}$). Therefore, HR50 reflects cardiac response to a standard low-intensity physical exertion, and the change in HR50 with endurance training is a measure of improvement in cardiac function. On the other hand, HR60 provides information about cardiac performance relative to one's maximal capacity. Against this background, it would not be surprising if these two traits were affected by both common as well as trait-specific sets of genes.

It is a common feature in genetic studies that QTLs affecting a given trait may vary across ethnicities, and there are several potential reasons for this observation. First, a true ethnicity-specific mutation, DNA sequence variant, or haplotype may affect the trait variance in only one ethnic group. Second, an ethnicity-specific gene-environment interaction effect, i.e., an environmental factor that promotes or inhibits the expression of a specific DNA sequence variant effect on a trait of interest may be present in one ethnic group but not in another. Third, differences in phenotype means and distributions between ethnic groups may reflect differences in the physiological pathways and, consequently, set of genes that regulate and/or modify the trait values. For example, mean baseline HR50 was higher in blacks than in whites, whereas mean baseline HR60 tended to be higher in whites than in blacks. Finally, differences in sample sizes, family structures, and other study design-related features could contribute to differences in observed QTLs.

In summary, we have reported the first genome-wide scan for HR50 and HR60 at baseline and in response to 20-wk of endurance training. The strongest evidence of linkage was detected with baseline HR50 on chromosome 18q in white subjects. In addition, evidence of linkage for ΔHR50 was identified on chromosome 2q33 and for ΔHR60 on chromosome 18q. We also identified a QTL on chromosome 10q25 for baseline HR50 and HR60 in black subjects. An exploration of two candidate genes of this QTL yielded an association between an ADRB1 SNP, Arg389Gly, and baseline HR50 in blacks. These genomic regions should be further explored to identify the genes and mutations that contribute to the regulation of exercise heart rate.

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