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Effect of Endothelin 1 Genotype on Blood Pressure Is Dependent on Physical Activity or Fitness Levels

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Abstract—Contributions of the DNA sequence variation at the endothelin 1 locus to the risk of hypertension and to endurance training–induced changes in blood pressure were investigated in the Aerobics Center Longitudinal Study and the Health, Risk Factors, Exercise Training and Genetics Family Study cohorts. We identified 586 normotensive control subjects and 607 incident hypertensive case subjects from the Aerobics Center Longitudinal Study cohort (all whites) who were normotensive and healthy at their first clinic visit. The case subjects were diagnosed with hypertension during an average follow-up of 9.5 years, whereas the control subjects remained normotensive. The allele and genotype frequencies of 5 endothelin 1 haplotype tagging single nucleotide polymorphisms did not differ significantly between the case and control subjects. However, we observed a significant ($P=0.0025$) interaction between the endothelin 1 rs5370 (G/T; Lys198Asn) genotype and cardiorespiratory fitness level on the risk of hypertension: among low-fit subjects, the rs5370 minor allele (T; 198Asn) was associated with higher risk of hypertension (odds ratio: 1.95; 95% CI: 1.36 to 2.81; $P=0.0003$), whereas the risk did not differ among genotypes in high-fit subjects. In the white Health, Risk Factors, Exercise Training and Genetics subjects (N=480), the rs5370 T allele was associated with blunted systolic blood pressure ($P=0.0046$) and pulse pressure ($P=0.0016$) responses to a 20-week endurance training program. The Lys198Asn variant of the endothelin 1 locus is associated with blood pressure phenotypes in whites. However, the expression of the genotype effect is modulated by physical activity or cardiorespiratory fitness level. Our study provides an illustrative example of how physical activity and fitness level modifies the associations between a candidate gene and outcome phenotype. **(*Hypertension*. 2007;50:1120-1125.)**

Key Words: genotype ■ exercise training ■ cardiorespiratory fitness ■ gene-environment interaction ■ HERITAGE Family Study ■ HYPGENE Study

Regular physical activity and a moderate-to-good level of cardiorespiratory fitness are key components in the prevention of hypertension and in the reduction of the comorbidities associated with hypertension. Although it is generally accepted that regular physical activity can lower blood pressure, there is great heterogeneity in terms of the magnitude of reduction across controlled exercise training studies.[1–3] In addition, there is considerable interindividual variation in the blood pressure responsiveness to endurance training within studies, and data from twin and family studies have shown that there is a significant genetic component affecting the variability in training responses.[4,5]

Endothelin 1 (EDN1) is a potent vasoconstrictor and, consequently, a key regulator of blood pressure. The endothelin family consists of 3 polypeptides encoded by separate genes located on chromosomes 6p24.1 (EDN1), 1p34 (endothelin 2), and 20q13.2 to 13.3 (endothelin 3). Although structurally and functionally similar, the expression patterns of the 3 endothelins vary considerably. EDN1 is expressed in several tissues, including endothelial cells and cardiomyocytes, whereas the expression of endothelin 2 and endothelin 3 seems to be focused on the gastrointestinal tract and on neuronal cells, respectively.[6,7] Only EDN1 is expressed constitutively on vascular endothelium and thereby affects vasomotor tone.[8] DNA sequence variations in the EDN1 gene locus have been reported to be associated with blood pressure levels and hypertension in some populations.[9–11] An interesting finding of previous studies exploring the relationships among hemodynamic traits and the EDN1 genotype is the seemingly more pronounced association in overweight or obese individuals.[9–12]

Increased shear stress because of enhanced blood flow has been proposed as a major mechanism for the blood pressure–

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lowering effect of exercise training.[13–15] Although high shear stress has been shown to inhibit EDN1 expression in endothelial cells,[6] there are few data available on the interactions among the EDN1 locus DNA sequence variation, exercise training, and cardiorespiratory fitness on blood pressure phenotypes. The purpose of the present study was to investigate these interactions in 2 large cohorts: endurance training-induced changes in blood pressure were targeted in the HEalth, RIsk factors, exercise Training And GEnetics (HERITAGE) Family Study and the risk of hypertension was focused on in the Genetics, Fitness, Obesity and Risk of Hypertension (HYPGENE) Study.

Methods

Subjects

The HERITAGE Family Study cohort consists of 493 white subjects (240 males and 253 females) from 99 nuclear families and 270 black subjects (90 males and 180 females) from 114 family units. The study design and inclusion criteria have been described previously.[16] To be eligible, the individuals were required to be sedentary but in good health, ie, free of diabetes, cardiovascular diseases, or other chronic diseases that would prevent their participation in a 20-week endurance exercise training program. The study protocol had been approved by each of the institutional review boards of the HERITAGE Family Study research consortium. Written informed consent was obtained from each participant.

The HYPGENE cohort is based on the Aerobics Center Longitudinal Study database.[17] All of the HYPGENE subjects included in this report are whites. All of the eligible Aerobics Center Longitudinal Study subjects for the HYPGENE Study were healthy with resting blood pressure $<$140/90 mm Hg at their first clinic visit and were required to have $\geq$2 clinic visits with a minimum of 1 year apart. Case subjects developed hypertension during the follow-up, defined as physician-diagnosed hypertension with medication to lower blood pressure, or resting systolic blood pressure (SBP) of $\geq$140 mm Hg, and/or resting diastolic blood pressure (DBP) of $\geq$90 mm Hg on a follow-up clinic visit. Control subjects remained normotensive and otherwise healthy throughout the follow-up period. The HYPGENE Study protocol has been approved annually by the institutional review boards of the Pennington Biomedical Research Center and the Cooper Institute. Written informed consent was obtained from each participant.

Exercise Training Program

The exercise intensity of the 20-week training program of the HERITAGE Family Study was standardized for each participant based on the heart rate (HR)-oxygen consumption (V$\dot{O}$) relationship measured at baseline.[18] During the first 2 weeks, the subjects trained at an HR corresponding with 55% of the baseline maximal oxygen consumption[2] for 30 minutes per session. Duration and intensity of the training sessions were gradually increased to 50 minutes and the HR associated with 75% of the baseline maximal oxygen consumption, respectively, which were then sustained for the last 6 weeks. Training frequency was 3 times per week, and all of the training was performed on cycle ergometers in the laboratory. Trained exercise specialists supervised all of the exercise sessions.

Phenotype Measurements

In the HERITAGE Family Study, both resting and exercise blood pressures were measured using Colin STBP-780 automated units, and the recordings were confirmed by technicians wearing headphones.[19] Submaximal exercise blood pressure (SBP50 and DBP50) was measured during 2 cycle ergometer tests, both before and after training in a relative steady state after 8 to 12 minutes at a constant power output (50 W). Pulse pressure (PP; resting PP and submaximal PP [PP50]) was calculated as a difference between SBP and DBP (SBP−DBP). HR (resting HR and submaximal HR [HR50]) was recorded by electrocardiography.

In the HYPGENE Study, cardiorespiratory fitness was assessed by a maximal exercise test following a modified Balke protocol.[17,20] Time-to-completion on the treadmill was used to estimate maximal metabolic equivalents (METs) using the following formula: METs=[1.44$\times$(minutes on treadmill)+14.99]/3.5.[21] Resting blood pressure was auscultated as the first and fifth Korotkoff sounds according to a standard sphygmomanometer protocol.[22] Stature and body mass were measured using standardized protocols, and body mass index (BMI) was calculated by dividing body mass (kilograms) by stature squared (meters squared) in both studies.

Genotyping

The EDN1 single nucleotide polymorphisms (SNPs) were selected from the National Institute of Environmental Health Sciences SNP resequencing database using the SNP spectral decomposition method.[23] Five haplotype tagging SNPs (rs2070699, rs5369 [Glu106Glu], rs5370 [Lys198Asn], rs4714383, and rs9296345) were selected for genotyping. These tagging SNPs explained $\approx$86% of the total DNA sequence variation in the National Institute of Environmental Health Sciences resequencing data set.

Genotyping of the EDN1 SNPs was done by the primer extension method with fluorescence polarization detection (PerkinElmer Inc). Details for PCR conditions and primer sequences are available on request. Haplotypes were constructed with Merlin software in the HERITAGE Family Study[24] and with Phase software (version 2.1) in the HYPGENE Study.[25,26]

Statistical Analyses

In the HERITAGE Family Study, baseline blood pressure phenotypes were adjusted for age, sex, and BMI, and blood pressure training responses were adjusted for age, sex, baseline BMI, and baseline value of the BP phenotype. The associations between *EDN1* markers and blood pressure phenotypes were analyzed using variance components and the likelihood ratio test–based total association model of the QTDT software package.[27] The model uses a variance-components framework to combine phenotypic means and the additive genetic, residual genetic, and residual environmental variances from a variance-covariance matrix into a single likelihood model.[27] The identity-by-descent allele sharing estimates for the QTDT analyses were generated with Merlin software.[24]

In the HYPGENE Study, logistic regression modeling was used to test the contribution of the EDN1 SNPs, as well as the SNP-by-fitness and SNP-by-BMI interactions to the risk of hypertension. The common allele homozygotes were used as the reference group for each SNP. All of the models included baseline age, sex, cardiorespiratory fitness, BMI, and follow-up time as covariates. In the SNP-by-fitness and SNP-by-BMI interaction models, subjects were categorized into low- and high-fitness groups based on sex-specific medians of maximal METs (12.5 METs in men and 10.25 METs in women) and low- and high-BMI groups based on sex-specific medians of BMI (24.9 kg/m^2 in men and 21.4 kg/m^2 in women).

Because multiple SNPs were used for the association studies, we applied a multiple testing correction proposed by Nyholt.[23] Briefly, the method uses spectral decomposition of matrices of pairwise linkage disequilibriums (r) to estimate variance of eigenvalues. The effective number of independent SNPs can be calculated based on the ratio of observed eigenvalue variance and its maximum. The effective number of SNPs can then be used to adjust the standard α level (eg, 5%). In our study, the corrected threshold for statistical significance was set to $P<0.0127$ for analyses with individual SNPs.

Results

Basic characteristics of the HERITAGE Family Study and the HYPGENE Study subjects are presented in Tables 1 and 2, respectively. The allele and genotype frequencies and the pairwise linkage disequilibria among the SNPs are summarized in Table S1 (available at http://hyper.ahajournals.org).

Table 1. Basic Characteristics of the HERITAGE Family Study Subjects

	Blacks		Whites	
Phenotype	Men	Women	Men	Women
Age, y	34.6 (12.4)	33.2 (11.4)	36.6 (15.0)	35.0 (14.1)
BMI, kg/m^2	27.3 (5.2)	28.2 (6.3)	26.7 (4.9)	25.0 (4.9)
Resting SBP, mm Hg	124.5 (10.1)	122.3 (13.1)	120.4 (10.8)	112.7 (9.9)
Resting DBP, mm Hg	72.9 (7.4)	72.6 (8.9)	68.5 (9.1)	63.9 (7.0)
Resting PP, mm Hg	51.6 (8.0)	49.7 (8.6)	51.9 (8.9)	48.8 (7.4)
Resting HR, bpm	62.8 (8.7)	69.5 (7.9)	62.1 (8.3)	66.9 (8.7)
SBP at 50 W, mm Hg	154.6 (18.3)	155.3 (21.8)	146.4 (18.2)	142.9 (21.1)
DBP at 50 W, mm Hg	80.1 (10.5)	79.0 (11.4)	72.4 (11.4)	70.1 (11.0)
PP at 50 W, mm Hg	74.5 (14.2)	76.3 (17.3)	74.0 (13.4)	72.8 (15.3)
HR at 50 W, bpm	108.9 (11.8)	135.2 (16.5)	106.4 (11.6)	128.1 (15.4)

Values are mean (SD).

All 5 of the SNPs were in Hardy-Weinberg equilibrium both in HERITAGE blacks and whites and in HYPGENE case and control subjects (all whites).

HERITAGE Family Study

The haplotype construction revealed 14 and 15 haplotypes in whites and blacks, respectively. In both races, 11 haplotypes had frequency >1% (please see Table S2 for details). Sedentary-state blood pressure phenotypes were not associated with the EDN1 haplotypes (data not shown). However, exercise training–induced changes in SBP50 and PP50 in whites and in HR50 in blacks showed significant global associations with the haplotype (Table 3). In allele-specific analyses, haplotypes 1, 3, 10, and 11 were associated with PP50 and SBP50 training responses. Haplotype 1 was associated with the greatest reductions in PP50 and SBP50, whereas carriers of haplotypes 3, 10, and 11 showed blunted training responses in whites. In single SNP analyses, markers rs5370 and rs4714383 were significantly associated with SBP50 and PP50 training responses (Figure 1 and Table S3). A closer inspection of the haplotypes revealed that these 2 markers characterized the alleles that were associated with SBP50 and PP50 training responses. The majority of the T alleles of the rs5370 locus were contained in haplotypes 10 and 11, whereas haplotypes 1 and 3 covered most of the G alleles. The only difference between haplotypes 1 and 3 was the allele present at the rs4714383 locus. Thus, the rs4714383 T allele defined a phenotypically distinct (low SBP50 and PP50 responses) subgroup among the rs5370 G/G homozygotes (otherwise high responders; Figure 1). The haplotype of rs5370 and rs4714383 explained 2.6% and 3.5% of the variance in SBP50 and PP50 training responses, respectively, whereas contribution of the individual SNPs ranged from 0.8% to 1.7%.

Table 2. Baseline Characteristics of the HYPGENE Study Subjects

Phenotype	Case Subjects	Control Subjects
No. of subjects	607	586
Sex, male/female, n	501/106	440/146
Age, mean (SD), y	43.3 (9.2)	42.6 (8.9)
BMI, mean (SD), kg/m^2	25.1 (3.2)	24.1 (3.1)
Maximal METs, mean (SD)	11.7 (2.1)	12.3 (2.0)
Resting SBP, mean (SD), mm Hg	117.3 (8.7)	110.6 (9.3)
Resting DBP, mean (SD), mm Hg	77.6 (6.0)	73.8 (6.9)
Follow-up, mean (SD), y	8.7 (6.4)	10.2 (7.0)

HYPGENE Study

There were no differences in the EDN1 SNP allele and genotype frequencies between the case and control subjects. However, 2 SNPs (rs2070699 and rs5370) showed significant interactions with cardiorespiratory fitness on the risk of hypertension (Table 4). Both SNPs were associated with the hypertension risk only in subjects with a low cardiorespiratory fitness level (maximal METs below sex-specific median). The G/T heterozygotes and the T allele homozygotes in the rs5370 locus showed 1.93 (95% CI: 1.32 to 2.81) and 2.17 (95% CI: 0.86 to 5.47) times greater risk of hypertension than the G/G homozygotes, whereas the minor allele (A) of the rs2070699 locus was associated with a lower hypertension risk (Figure 2). Further analyses of haplotypes constructed from rs5370 and rs2070699 revealed that the higher hypertension risk associated with the rs5370 T allele was particularly marked among the C/C homozygotes of the rs2070699 (Table S4). The haplotype analyses also revealed that the lower risk among the rs2070699 A allele carriers seen in the single SNP analysis reflects, in large part, the fact that all of the T/T homozygotes and a large portion of the G/T heterozygotes of the rs5370 were among the rs2070699 reference group (C/C homozygotes). All of the associations were independent of baseline BMI, and there was no evidence of SNP-by-BMI interactions on the risk of hypertension (Table 4).

Discussion

The novel finding of the present study is that the associations between DNA sequence variation in the EDN1 locus and blood pressure phenotypes are modulated by physical activity or cardiorespiratory fitness levels. Both in the HERITAGE Family Study and in the HYPGENE Study, the minor allele

Table 3. Associations Between the EDN1 Haplotypes and Endurance Training–Induced Changes in Submaximal Exercise (50 W) Blood Pressure Phenotypes in Whites and Blacks of the HERITAGE Family Study

	Whites				Blacks			
Haplotype	ΔSBP	ΔDBP	ΔPP	ΔHR	ΔSBP	ΔDBP	ΔPP	ΔHR
Global	0.0199	0.4066	0.0001	0.9315	0.8728	0.2724	0.4752	0.0357
1	0.0141	0.7773	0.0017	0.2207	0.9203	0.2161	0.2524	0.8415
2	0.7184	0.5023	0.5220	0.9203	...	...	...	...
3	0.0605	0.1016	0.0015	0.6714	0.6547	0.8875	0.5271	0.2560
4	0.8065	1.0000	1.0000	0.4201	...	...	...	...
5	0.0453	0.2017	0.1949	0.4096	...	...	...	...
6	...	...	...	...	0.1049	0.6033	0.1604	0.4310
7	0.5169	0.2616	0.9203	0.6714	0.9203	0.0469	0.1277	0.7518
8	0.6033	0.6101	0.7642	0.543	0.2943	0.3272	0.5485	0.0828
9	0.3897	0.3994	0.6547	0.5657	0.4237	0.7642	0.2987	1.000
10	0.0880	0.3247	0.0104	1.000	0.3510	0.9203	0.3078	0.4583
11	0.0164	0.5598	0.0168	0.4976	0.7083	0.4543	0.8231	0.0096
12	0.5656	0.0954	0.7290	0.2674	1.000	0.2269	0.3032	0.1003
13	...	...	...	...	0.008	0.1573	0.0251	0.7518
14	0.2636	0.5598	0.1580	0.7083	1.000	0.5071	0.4930	0.5902
15	0.6629	0.3897	0.3102	0.3009	0.2694	0.1563	0.8065	0.1692
16	0.9203	0.3455	0.5376	0.8415	0.0547	0.6985	0.0954	0.5430
17	...	...	...	...	0.0869	0.3272	0.2418	0.9203
18	...	...	...	...	0.8625	0.8625	0.7184	0.6315

··· indicates no data.

of the rs5370, which induces a lysine-to-asparagine substitution in codon 198 (Lys198Asn) of EDN1, was associated with a less favorable blood pressure outcome. In HERITAGE, 20 weeks of endurance training in previously sedentary whites lowered steady-state submaximal exercise SBP and PP less in the rs5370 T allele carriers than in the common allele homozygotes. Likewise, in the low-fit subjects of the HYPGENE cohort, the T allele was associated with a 2-fold risk of hypertension as compared with the G/G homozygotes, whereas no genotype effect was observed in the high-fit subjects. The same allele has been reported previously to be associated with elevated resting DBP in obese Japanese subjects,[9] elevated resting and exercise SBP in overweight whites,[10] increased BP reactivity to a video game challenge,[12] and with increased in vitro vascular reactivity.[28] Therefore, our novel discovery of a significant relationship between rs5370 and SBP responsiveness to endurance training is concordant with existing data on other hemodynamic phenotypes, ie, the T allele is associated with a less favorable hemodynamic profile.

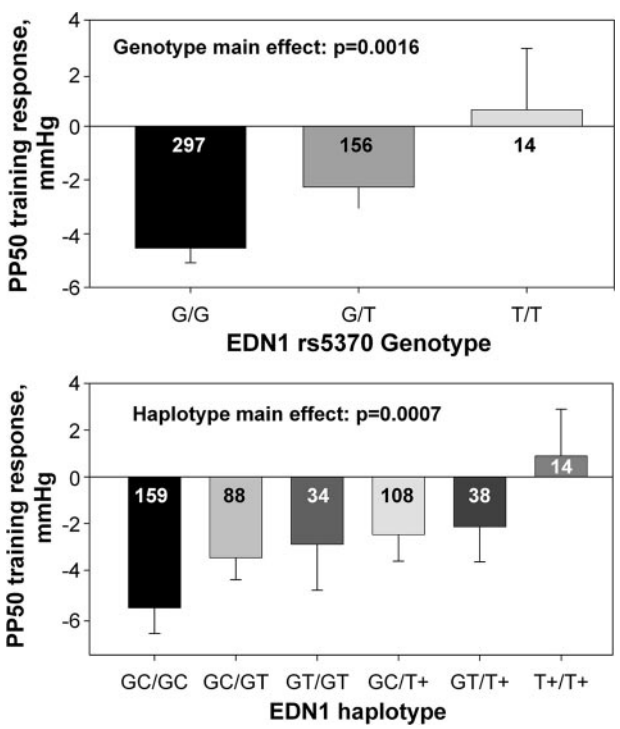

Figure 1. Associations between PP50 training response and EDN1 rs5370 genotype (top) and rs5370 and rs4714383 haplotype (bottom) in the whites of the HERITAGE Family Study.

The functional significance of rs5370 is not clear at this time. Indeed, although the G-to-T transversion induces a nonsynonymous amino acid change in codon 198 of the prepro-EDN1 molecule, codon 198 is cleaved out from the biologically active EDN1 peptides. As such, alternative causal mechanisms, such as an effect on mRNA stability, must be considered. In both of our studies, a 2-SNP haplotype composed of rs5370 and an additional SNP (rs2070699 in HYPGENE and rs4714383 in HERITAGE) seemed to further fine tune the associations with blood pressure phenotype. This pattern suggests that the rs5370 may tag another functional SNP or a group of functional sequence variants. This and related hypotheses need to be tested in future studies using resequencing in a large group of informative subjects.

Table 4. Associations Between the EDN1 SNPs and the Risk of Hypertension in the HYPGENE Study

SNP	Map	SNP Main Effect	SNP-by-Fitness Interaction	SNP-by-BMI Interaction
rs2070699	12 400 758	0.7720	0.0006	0.7766
rs5369	12 402 244	0.9720	0.9360	0.7777
rs5370	12 404 241	0.2240	0.0025	0.3720
rs4714383	12 405 468	0.6640	0.2090	0.8303
rs9296345	12 406 319	0.6003	0.9770	0.6377

P values from logistic regression models are given for the SNP main effects and the SNP-by-fitness and SNP-by-BMI interaction terms (adjusted for baseline age, BMI, cardiorespiratory fitness, and follow-up time).

The effect of increased laminar shear stress caused by exercise on endothelial function is well documented.[14,15,29,30] The majority of the research in this area has focused on the activation of endothelial NO synthase gene expression and, by extension, increased NO production. In addition to vasodilatory substances, such as NO, the net peripheral resistance and, consequently, BP are also influenced by compounds promoting vasoconstriction, the most potent being the EDN1. High shear stress has been shown to decrease EDN1 expression, but data on the effects of exercise on plasma and tissue endothelin levels or on endothelin gene expression are scarce. Two studies in small groups of young and elderly Japanese subjects have reported significant reductions in plasma EDN1 levels after 2 to 3 months of endurance training.[31,32] Also, EDN1 mRNA levels in the heart were significantly lower in exercise-trained (12 weeks) spontaneously hypertensive rats than in sedentary control animals.[33] However, in normotensive Wistar-Kyoto rats, EDN1 expression in the heart either tended to decrease or increase after exercise training.[34–36] Thus, the overall effect of exercise training on plasma and tissue levels of EDN1 has not yet been fully elucidated.

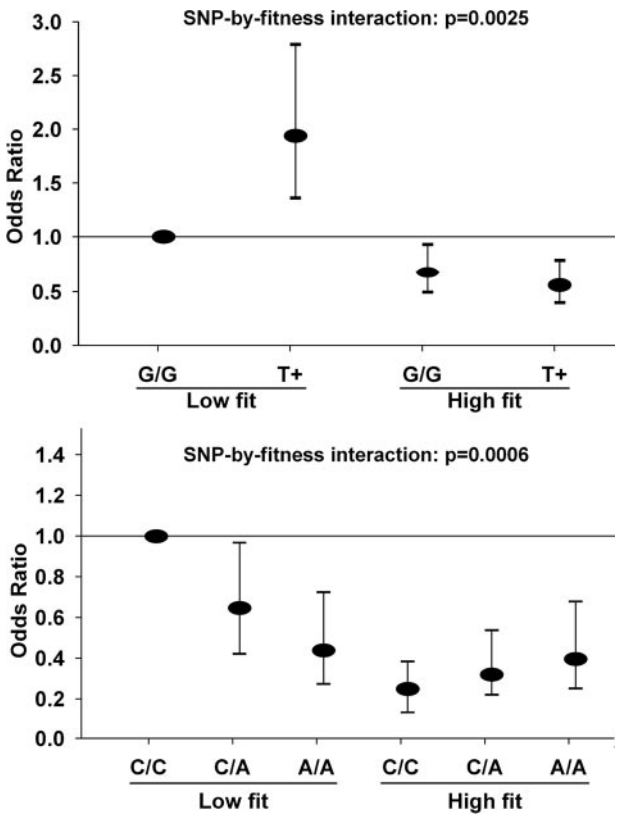

Figure 2. Genotype-by-fitness interactions on the risk of hypertension with SNPs rs5370 (top) and rs2070699 (bottom) in the HYPGENE Study.

Lack of replication has been cited frequently as a major problem in genetic studies of complex, multifactorial traits. Inadequate statistical power and, therefore, inflated type 2 error rate are often credited for the low replication rates. However, differences in behavioral and physiological characteristics of the subjects across studies are other potential explanations. Our study provides 2 excellent examples of how physical activity and cardiorespiratory fitness levels modify the associations between a candidate gene and outcome phenotype in whites. Some previous studies have reported previously that the association between the EDN1 polymorphisms and blood pressure is modified by body weight, ie, the associations are observed only in overweight or obese subjects.[9–12] We did not observe evidence for such modification, but it must be kept in mind that both of our cohorts were normal weight on average and, as such, had limited power to detect gene-obesity interactions. Also, there are several possibilities as to why we did not observe the same associations in blacks as we did in whites. It is possible that the EDN1 locus has less contribution in blacks, and the greater baseline blood pressure and BMI levels in blacks may alter the physiological pathways contributing to exercise training–induced blood pressure changes. It is also possible that the tagging SNPs did not capture the same degree of information of the overall haplotype structure in blacks as they did in whites. Finally, if the EDN1 locus has only a minor effect on blood pressure traits in blacks, it is possible that our sample size is not large enough to detect such a small effect size.

Perspectives

Our results suggest that DNA sequence variation in the EDN1 gene locus is associated with blood pressure phenotypes in whites. However, the expression of the genotype effect is modulated by physical activity or cardiorespiratory fitness level. These data provide an illustrative example of how physical activity and fitness level modify the associations between a candidate gene and outcome phenotype. They also emphasize the importance of incorporating key behavioral and physiological traits in genetic association studies to better understand how the interactions between DNA sequence variants and nongenetic factors affect multifactorial phenotypes, such as blood pressure.

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Disclosures

None.

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