A Tyrosine Hydroxylase Microsatellite and Hemodynamic Response to Stress in a Multi-Ethnic Sample of Youth

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Objective: Behavioral stress is believed to have an impact on cardiovascular health. As the rate-limiting enzyme in the pathway for catecholamine synthesis, tyrosine hydroxylase is a candidate gene for variability in cardiovascular function. The aim of this study was to determine whether a relationship exists between a tyrosine hydroxylase microsatellite and resting hemodynamic function, and/or hemodynamic responsivity to laboratory stress.

Design: Subjects underwent 2 laboratory stressors: a video game challenge and a social competence interview.

Setting: The stressors were administered in a laboratory setting.

Participants: Subjects were 292 10- to 20-year-old normotensive African-American and European-American twin pairs.

Main Outcome Measures: Blood pressure (BP) and heart rate (HR) were measured at rest and in response to the stressors.

Results: Chi-square analyses using re-sampling to account for the twin design indicated that allele and genotype frequencies were significantly different between European Americans and African Americans ($P \leq .0001$). Analyses of variance indicated that the 184 and 199 bp alleles were associated with an attenuation of the hemodynamic response to stress with increasing age ($P \leq .003$, $P \leq .002$, respectively), while the 188 bp allele was associated with a higher resting systolic blood pressure (SBP) ($P \leq .02$), and greater hemodynamic response to stress with increasing BMI ($P \leq .02$).

Conclusions: This study showed that in a multi-ethnic sample of normotensive adolescents, specific alleles of this tyrosine hydroxylase microsatellite were associated with protective or deleterious cardiovascular effects with subjects at rest and responding to stress. (*Ethn Dis.* 2003;13:186–192)

Key Words: Blood Pressure, Adolescents, Cardiovascular Disease, Hypertension, Genetic

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Introduction

Contributions from a number of fields suggest that stress has an overall negative impact on cardiovascular health.[1–3] The rapid utilization of catecholamines by the sympathetic nervous system (SNS) is believed to be a primary neuro-hormonal mechanism mediating an organism's stress response. Both plasma and urinary catecholamine levels (ie, norepinephrine, dopamine, and epinephrine) have been measured extensively in the context of cardiovascular diseases (CVD), particularly for blood pressure (BP) control among hypertensive subjects and matched controls.[4] The inconsistent findings of these studies are likely due to the secondary effects of hypertension's involvement in multiple organ systems. The interpretation of catecholamine levels is further obscured by differential catecholamine uptake, release, and clearance at SNS nerve endings of various vascular beds.[4] Despite these difficulties, animal models exposed to acute or long-term stress, such as immobilization[5,6] and cold,[7–10] have demonstrated increased gene expression of tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine biosynthesis, making the regulation of TH potentially important for the production of catecholamines in response to stress.

Twin studies have indicated that SNS activation to acute stress is mediated, in part, by genetic factors.[11,12] The TH gene possesses an informative microsatellite marker consisting of a tetranucleotide repeat $(TCAT)_{5–11}$ within intron 1.[13] Deletion of the entire repeat has shown that it functions as a transcriptional enhancer.[14] This suggests that the microsatellite may have a direct influence on gene expression; however, biochemical analyses of the various alleles have not yet been reported. With respect to human phenotypic assessments, the available studies are few and contradictory. Sharma et al[15] found a weak prevalence of the 196 bp allele, accompanied by lower plasma norepinephrine, among normotensive subjects, whereas Wei et al[16] found the 196 bp allele to be associated with significantly higher norepinephrine levels.

Increased vasoconstrictor-mediated responsivity to stress in youth and adults has been found to segregate with standard risk factors for future cardiovascular disease, including being male, of African-American ethnicity, and having a positive family history of hypertension.[17,18] Responsivity to laboratory stress is predictive of future hypertension in adults,[19] and of preclinical markers for CVD, including increased resting[20] and ambulatory[21,22] BP, and increased left ventricular mass,[23] in youth. Being overweight has also been shown to be related to higher resting hemodynamics[24] and ambulatory BP,[22] although not all studies have found an association between body mass and stress reactivity.[24] Of particular interest is the association found between central adiposity and increased reactivity,[25] even after correcting for overall and peripheral adiposity.[26]

In order to address the possible underlying impact of the TH gene on moderating stress-induced disease pathways, we genotyped this TH microsatellite in a cohort of youths while mea-

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The rapid utilization of catecholamines by the sympathetic nervous system (SNS) is believed to be a primary neuro-hormonal mechanism mediating an organism's stress response.

suring their cardiovascular responsivity to laboratory stress. The aim of this study was to test the hypothesis that one or more specific TH $(TCAT)_n$ alleles would be associated with: 1) resting hemodynamic function; and 2) hemodynamic responsivity to laboratory stress.

METHODS

Study Population

Participants were twin pairs recruited for a longitudinal study of the heritability of the bio-behavioral antecedents of hypertension.[27] Pursuant to Medical College of Georgia guidelines, consent was obtained from 292 twin pairs (197 monozygotics, 86 same-sex dizygotics, 9 different sex dizygotics; 119 AA pairs, 173 EA pairs) ranging from 10 to 20 years of age. The descriptive statistics for individuals by ethnicity and sex subgroups are presented in Table 1.

Protocol

Prior to the stress protocol, subjects' height and weight were measured. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Two sets of electrodes were placed on each side of the subject's neck and chest for noninvasive thoracic bioimpedence measurements of heart rate (HR) (NCCOM-3, Model 6; Bo-Med Medical Manufacturing Ltd., Irvine, Calif). A properly fitted blood pressure cuff was placed on the subject's right arm, and systolic (SBP) and diastolic (DBP) blood pressures were measured by a Dinamap Vital Signs Monitor (Model 1846SX; Critikon, Inc., Tampa, Fla), which has been validated for use during reactivity evaluations.[28,29] HR values were averaged from measurements made at each QRS complex during the inflation of the Dinamap for BP measurements.

Subjects were asked to relax as completely as possible on a comfortable bed in a quiet, temperature-controlled room. From the adjacent observation room, separated by a one-way mirror, resting hemodynamics were measured 11, 13, and 15 minutes after the rest period had begun. The first behavioral stressor was a social competence interview, for which the subjects selected a stressful situation from a list of potential stressors derived from school, family, friends, work, money, and neighborhood.[30] A structured interview assisted each subject in re-experiencing the stressful situation. Hemodynamic readings were recorded at 0, 2, 4, 6, 8, and 10 minutes with the subject in a supine position. The second behavioral stressor was a car-driving video game challenge which consisted of a 5 minute car-driving simulation, made more realistic by the use of a virtual reality headset.[31] While lying on a bed, subjects were briefly familiarized with the game, then instructed to drive a Porsche 911 as fast as they could in order to catch a Ferrari. Hemodynamics were recorded at 0, 1, 3, and 5 minutes after starting the game. The video game and social competence interview were presented in a counterbalanced manner. A recovery period followed each stressor until SBP was within 5 mm Hg of the average of the baseline pressures recorded 13 and 15 minutes into the initial rest period. Recovery measures were taken every 2 minutes, for a maximum of 14 minutes. The reactivity for each stressor was calculated as the mean response score mi-

Table 1. Descriptive statistics (mean ± SD) by ethnicity and sex subgroups*

	Male *N*=265	Female *N*=319	*P* value	EA *N*=370	AA *N*=214	*P* Value
Age (y)	14.1 ± 2.3	14.2 ± 2.4	NS	14.3 ± 2.4	14.0 ± 2.3	NS
BMI (kg/m^2)	21.3 ± 4.7	21.5 ± 4.5	NS	21.3 ± 4.7	21.6 ± 4.5	NS
Resting hemodynamics						
SBP (mm Hg)	111.1 ± 9.7	106.8 ± 9.1	≤.0001	107.6 ± 9.3	110.8 ± 9.9	≤.001
DBP (mm Hg)	56.9 ± 6.0	58.5 ± 5.6	≤.005	56.9 ± 5.5	59.4 ± 6.0	≤.0001
HR (bpm)	67.6 ± 11.2	72.8 ± 11.5	≤.0001	70.9 ± 12.1	69.7 ± 10.9	NS
Reactivity hemodynamics						
SBP (mm Hg)	18.1 ± 8.3	15.5 ± 7.5	≤.005	17.3 ± 8.1	15.7 ± 7.6	NS
DBP (mm Hg)	15.0 ± 5.8	15.4 ± 6.1	NS	15.9 ± 5.9	14.2 ± 5.9	≤.005
HR (bpm)	14.9 ± 7.3	15.7 ± 7.8	NS	16.0 ± 7.7	14.1 ± 7.2	≤.05

EA=European American; AA=African American; SBP=systolic blood pressure; DBP=diastolic blood pressure; HR=heart rate.

* *P* values for age comparisons are from Wilcoxon's rank sums test, using one observation per family. *P* values for all other variables are from repeated measures ANOVA, using both observations per family.

nus the mean pre-stressor value. The mean resting SBP, DBP, and HR, and the mean of the 2 reactivity measures, were used as the dependent variables. The BP and HR responses in the 2 stressors were significantly correlated as follows: SBP $r = 0.36$ ($P<.0001$), DBP $r=0.23$ ($P<.0001$), and HR $r=0.24$ ($P<.0001$). Note that results of the aggregated scores were largely consistent with those of the stressors analyzed separately. Therefore, the BP and HR responses to the 2 stressors were aggregated in order to assess an overall propensity for responsivity to several types of behavioral stress, which, collectively, may better generalize to stress experiences in the natural environment, and to provide a more reliable representation of this propensity. The subjects received $75.00 as compensation for their participation.

Genotyping

The tyrosine hydroxylase microsatellite used in this study was one of 5 used to determine twin zygosity. The microsatellite was genotyped utilizing primers with fluorescent tags, as described by Becker et al.[32] The polymerase chain reactions (PCRs) were further optimized in order to develop a pentaplex reaction for higher throughput. Equimolar primer pairs were used at the following final concentrations: 0.05 μM THO, 0.07 μM TPOX, 0.32 μM FES/FPS, 0.16 μM F13A01, and 0.20 μM FGA. Other components were used as follows: 1× Taq Gold buffer (Perkin Elmer), 0.5 U Taq Gold enzyme (Perkin Elmer), 250 μM dNTPs, and 4.0 $MgCl_2$. Reactions were performed in a final volume of 10 μL, with approximately 1–10 ng of genomic DNA. The DNA was extracted from buccal swabs stored in STE buffer (0.1 M NaCl, 0.01 M Tris-HCl, 0.01 M EDTA, and 0.5% SDS), and affinity purified (QiaAmp DNA Blood Mini Kit; Qiagen). PCR was performed to amplify the fragment of interest in a GeneAmp 9600 thermal cycler (Perkin Elmer), starting with 10 minutes of denaturation at 95°C, followed by 30 cycles (15 seconds of denaturation at 94°C, 2 minute ramp to anneal at 54°C for 30 seconds, 1 minute extension at 72°C), and a final extension at 60°C for 30 minutes. The PCR products were separated on a 5% gel using an automated DNA sequencer (ABI Prism 377, Applied Biosystems, Foster City, Calif). Allele identities were determined with Genotyper software as 184, 188, 192, 196, 199, and 200 bp fragments. Allele identification was confirmed by sequence analysis. Although this microsatellite is a tetranucleotide, we, and others,[15] have identified an allele of 199 bp, which is distinct from the 200 bp allele.

Table 2. Allele frequencies (%) by ethnicity*

Allele (bp)	AA	EA
180	0.23	0.41
184†	15.42	23.51
188†	42.52	16.49
192†	20.33	10.27
196†	10.05	15.27
199†	10.51	33.51
200	0.23	0.54
203	0.47	0.00
204	0.23	0.00

AA=African American; EA=European American.
* $\chi^2=84.74$, $P\leq.0001$, $df=8$, from re-sampling analysis.
† Alleles for which association studies were performed.

Statistics

Frequencies of occurrence of each allele and of each genotype present in the sample were tabulated separately by ethnicity. Both siblings of each twin pair were included in these tabulations. The frequencies of occurrence of alleles and of genotypes were compared between

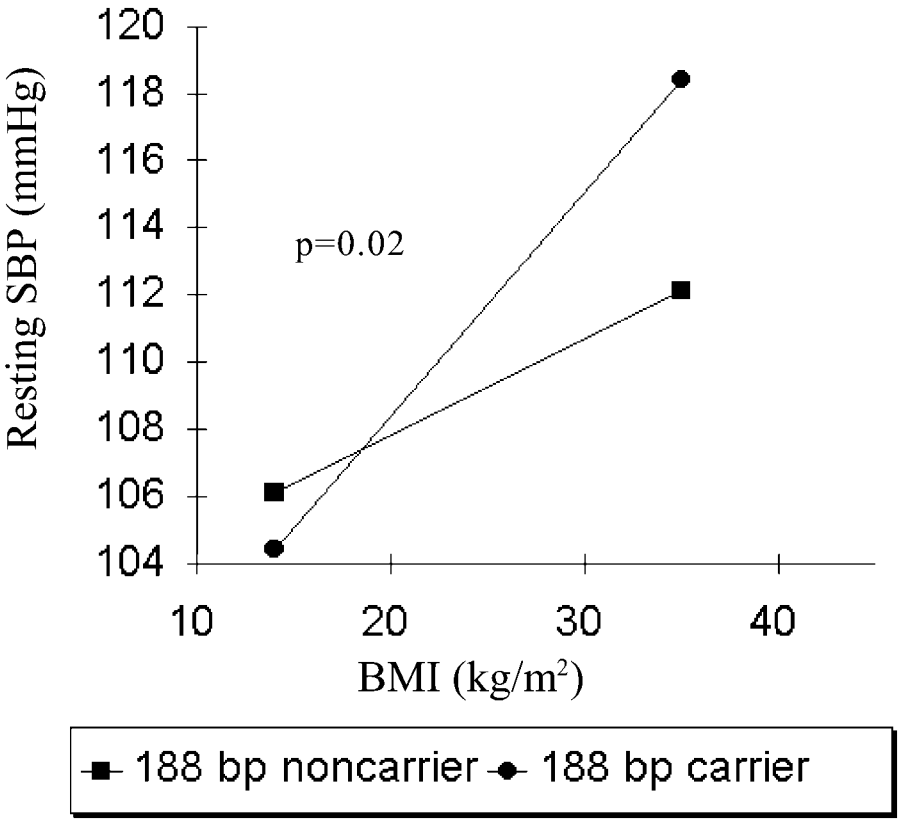

Fig 1. Regression of resting systolic blood pressure (SBP) on body mass index (BMI) for carriers and non-carriers of the 188 bp allele. *P* value is for difference between slopes

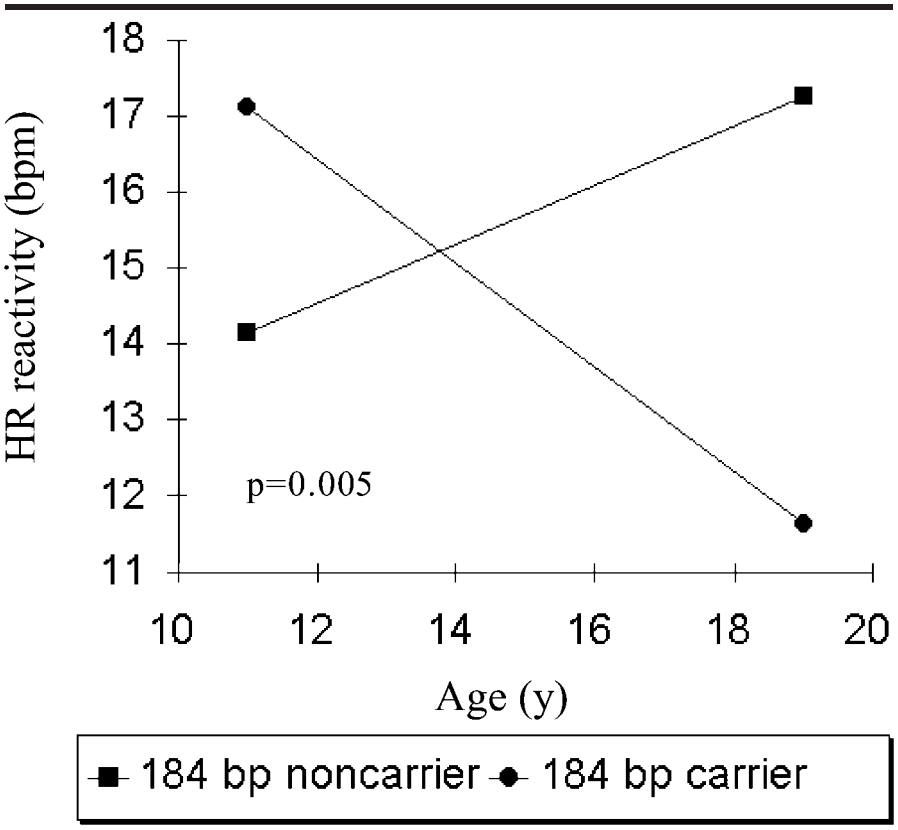

Fig 2. Regression of heart rate (HR) reactivity on age for carriers and non-carriers of the 184 bp allele. *P* value is for difference between slopes

ethnicities by chi-square test, incorporating a re-sampling technique to account for non-independence of observations on the twin pairs. The re-sampling was implemented by repeatedly tabulating the allele or genotype frequencies by ethnicity, using one sibling randomly selected from each family. From 1000 replications of this procedure, a table of median frequencies of occurrence of each allele or genotype in each ethnicity was constructed, and the distributions of frequencies were compared between the ethnic groups using the chi-square statistic.

Association studies were performed for alleles having a prevalence in the sample of greater than 5% for either ethnicity. Each study subject was categorized according to his or her carrier status for each allele identified in the analysis of frequencies. Analyses of associations of each of the alleles with SBP, DBP, and HR were performed using mixed model analyses of variance. Since the same alleles were identified in both ethnicities, all subjects were used in the analyses of allele associations. Carrier status, ethnicity, age, BMI, and all 2-factor interaction terms were included in the model. In order to account for correlations among observations of twin pairs, a term representing FAMILY was included in the model as a random effect. Zygosity was included as a grouping effect in the model, in order to allow separate estimates of intra-class correlation for monozygotic and for dizygotic twin pairs. The mixed model analysis was implemented using SAS® PROC MIXED (SAS Institute, Inc., Cary, NC, 1999–2001). When significant interaction terms were identified, the nature of the interaction effects was investigated by plotting regression lines for carriers and non-carriers, and then examining the slopes of these lines. Analyses of the genotypes were largely redundant with the allele association analyses, and are not included in this report.

RESULTS

Allele and Genotype Frequencies

Allele frequencies are presented in Table 2. The allele frequencies were significantly different ($P \leq .0001$) between EAs and AAs, and are similar to those previously reported in the literature.[13] The same 5 alleles were identified as having a prevalence of greater than 5% for each ethnicity; therefore, the association analyses were performed for the entire group, with ethnicity as a factor. Twenty-two genotypes were present in this cohort; the genotype frequencies were significantly different between EAs and AAs ($P \leq .0001$). For EAs, 5 of the genotypes had frequencies greater than 10% (ie, 184/196, 184/199, 188/199, 196/199, 199/199), while for AAs only 3 genotypes occurred at a rate of greater than 10% (ie, 184/188, 188/188, 188/192).

Association Studies for Resting Hemodynamics

Carrier status for the following bp alleles was investigated: 184, 188, 192, 196, and 199. No significant interactions of carrier status with ethnicity were observed for any of the tests. The results indicated a significant interaction between carrier status of the 188 bp allele and BMI for SBP ($P \leq .02$). Figure 1 shows that higher SBP was associated with higher BMI, and that the slope of this relationship was steeper for carriers of the 188 bp allele, compared to non-carriers.

Association Studies for Hemodynamic Reactivity to Stressors

The carrier status of the same alleles was investigated with respect to hemo-

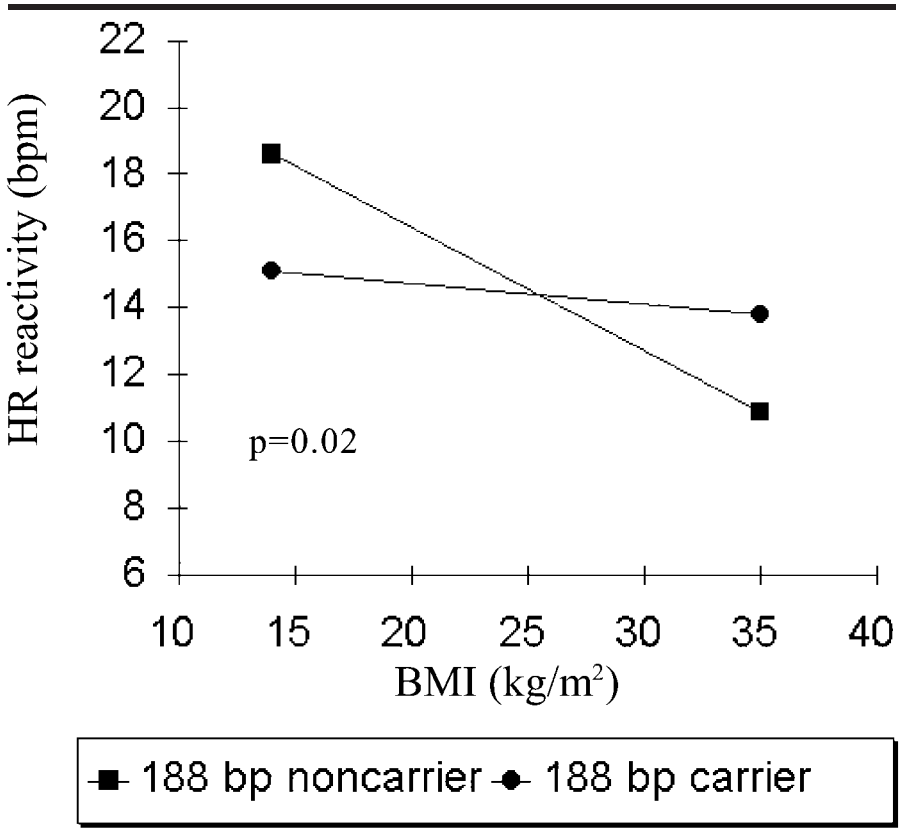

Fig 3. Regression of heart rate (HR) reactivity body mass index (BMI) for carriers and non-carriers of the 188 bp allele. *P* value is for difference between slopes

dynamic response to the stressors. There were no significant interactions of carrier status with ethnicity for any of the tests. Results indicated a significant interaction between carrier status of the 184 bp allele and age for HR reactivity (P=.005). Figure 2 shows that in carriers of the 184 bp allele, older age was associated with a lesser HR reactivity, while in non-carriers, older age was associated with a greater HR reactivity. A significant interaction was also found between carrier status of the 188 bp allele and BMI for HR reactivity (P=.02). Figure 3 shows that in non-carriers of the 188 bp allele, higher BMI was associated with a lesser HR reactivity, while little or no association was observed between BMI and HR reactivity in carriers. A significant interaction was observed between carrier status of the 199 bp allele and age for SBP (P=.002). Figure 4 shows that in non-carriers of the 199 bp allele, older age was associated with a greater SBP reactivity, while no association was found in carriers.

DISCUSSION

To the best of our knowledge, only one study has examined the relationship between the TH $(TCAT)_n$ microsatellite and hypertension in humans.[15] In this study, Sharma et al found that allele frequencies differed significantly between a group of 227 hypertensive subjects and 206 normotensive subjects, such that the 196 bp and 199 bp alleles were more prevalent in the normotensive and hypertensive groups, respectively. A multiple regression model using sex, age, and BMI as co-variates found no association between TH genotype and resting BP or HR in either the normotensive or hypertensive group.

The present study examined the relationship in youth between the TH $(TCAT)_n$ microsatellite and a wider range of hemodynamic variables, with subjects at rest, and responding to stressors. Our results indicated that: 1) carriers of the 188 bp allele, compared to non-carriers, tended to have a higher resting SBP with higher BMI, and greater HR reactivity with increasing BMI; 2) carriers of the 184 bp allele, compared to non-carriers, demonstrated a lesser HR reactivity with increasing age; and 3) carriers of the 199 bp allele, compared to non-carriers, had a lesser SBP reactivity with increasing age. The frequencies of these alleles were significantly different between AAs and EAs, with EAs having a greater frequency of the 184 and 199 bp alleles, compared to AAs, and AAs having a greater fre-

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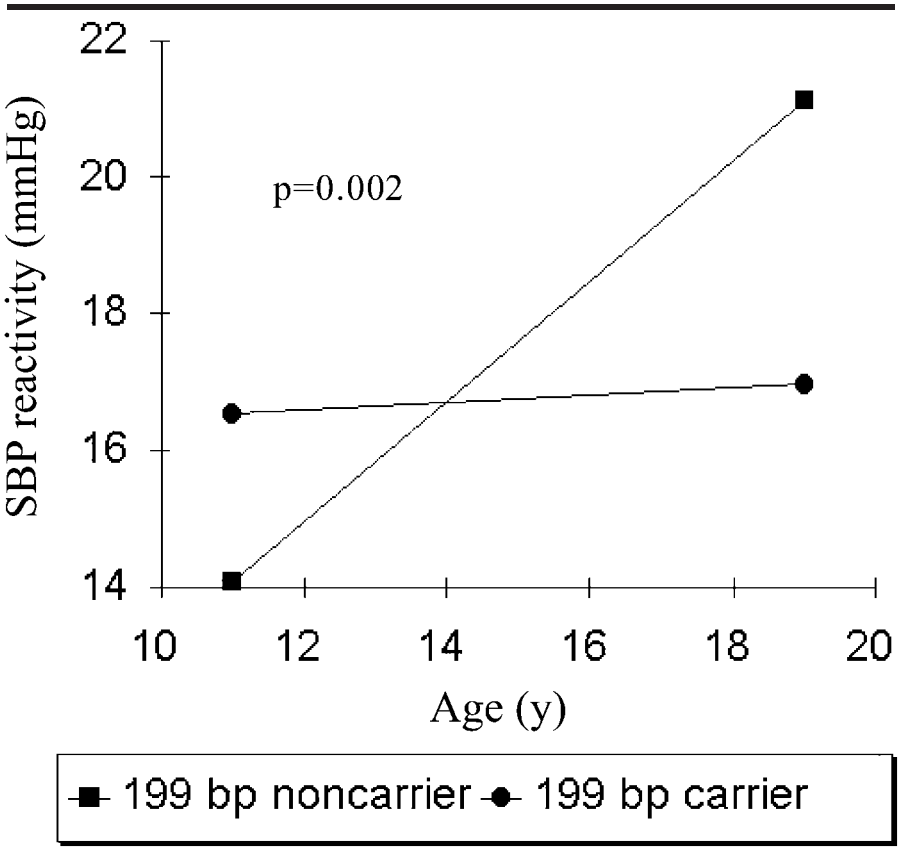

Fig 4. Regression of systolic blood pressure (SBP) reactivity on age for carriers and non-carriers of the 199 bp allele. *P* value is for difference between slopes

quency of the 188 bp allele, compared to EAs. Therefore, although the lack of an ethnicity by allele interaction indicated that the relationship between the alleles and the phenotypes did not differ between the 2 ethnicities, the higher prevalence of a specific allele within one ethnic group would make the allele's effects more important for that group. For example, at a frequency of 33% for EAs vs 11% for AAs, the effect of the 199 bp allele may be more important at a population level for EAs than for AAs. The same would be true for the greater frequency of the 188 bp allele in AAs.

It remains uncertain whether this particular TH microsatellite has any functional relevance, or whether the associations we found were due to another mutation in linkage disequilibrium with it. However, other microsatellite repeats have been implicated with a variety of diseases, such as Fragile X Syndrome[33] and at least 15 neurologic diseases.[34] Furthermore, in vitro research suggests that this TH microsatellite acts as a transcriptional enhancer element,[14] which means it may play a role in regulating TH gene expression. Both animal models and human studies have found acute and prolonged stress to be associated with increased production of catecholamines. Perhaps the TH gene is expressed differently, depending on environmental conditions; this study points to a gene-environment interaction of TH gene expression with age and BMI, considering that BMI is representative of the balance between caloric intake and expenditure in an individual.

In summary, the present study was novel in examining the relationship in youth between the TH $(TCAT)_n$ microsatellite and hemodynamics with subjects at rest, and responding to stress. The results indicated an association between this microsatellite and hemodynamics, such that the 184 and 199 bp alleles seemed to be protective by being associated with an attenuation of the hemodynamic response to stress with increasing age, while the 188 bp allele seemed to be deleterious by its association with a higher resting SBP and greater hemodynamic response to stress with increasing BMI. Future studies should address the role of this variant in modifying other components of the stress response (plasma catecholamines, microneurography for SNS activity, etc).

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Design and concept of study: Barbeau, Jackson, Treiber
Acquisition of data: Jackson, Treiber
Data analysis and interpretation: Barbeau, Litaker, Jackson
Manuscript draft: Barbeau, Litaker, Jackson, Treiber
Statistical expertise: Litaker
Acquisition of funding: Treiber
Administrative, technical, or material assistance: Barbeau, Jackson
Genetic analyses of results: Barbeau