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Adrenergic Polymorphism and the Human Stress Response

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Abstract

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis. Does common genetic variation at human *TH* alter autonomic activity and predispose to cardiovascular disease? We undertook systematic polymorphism discovery at the *TH* locus, and then tested variants for contributions to sympathetic function and blood pressure. We resequenced 80 ethnically diverse individuals across the *TH* locus. One hundred seventy-two twin pairs were evaluated for sympathetic traits, including catecholamine production and environmental (cold) stress responses. To evaluate hypertension, we genotyped subjects selected from the most extreme diastolic blood pressure percentiles in the population. Human *TH* promoter haplotype/reporter plasmids were transfected into chromaffin cells. Forty-nine single nucleotide polymorphisms (SNPs) and one tetranucleotide repeat were discovered, but coding region polymorphism did not account for common phenotypic variation. A block of linkage disequilibrium spanned four common variants in the proximal promoter. Catecholamine secretory traits were significantly heritable, as were stress-induced blood pressure changes. In the *TH* promoter, significant associations were found for urinary catecholamine excretion, as well as blood pressure response to stress. *TH* promoter haplotype #2 (TGGG) showed pleiotropy, increasing both norepinephrine excretion and blood pressure during stress. In hypertension, a case-control study (1266 subjects, 53% women) established the effect of C-824T in determination of blood pressure. We conclude that human catecholamine secretory traits are heritable, displaying joint genetic determination (pleiotropy) with autonomic activity and finally with blood pressure in the population. Catecholamine secretion is influenced by genetic variation in the adrenergic pathway encoding catecholamine synthesis, especially at the classically rate-limiting step, TH. The results suggest novel pathophysiological links between a key adrenergic locus, catecholamine metabolism, and blood pressure, and suggest new strategies to approach the mechanism, diagnosis, and treatment of systemic hypertension.

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Conflicts of Interest The authors declare no conflicts of interest.

Keywords

blood pressure; cold pressor test; heart rate; (TCAT)_n polymorphism in the first TH intron; tyrosine hydroxylase

Introduction

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis.^{1,2} Substantial loss of TH enzymatic activity as a consequence of rare inactivating mutations has profound consequences in humans,¹ as well as in mice with targeted ablation of the *TH* locus.³

The human *TH* locus also displays more common natural allelic variation, such as the tetranucleotide repeat (or microsatellite polymorphism) (TCAT)_n in its first intron.⁴ This microsatellite may be associated with essential hypertension.⁴ The spectrum of allelic variation at human *TH* is currently unknown; therefore, we undertook systematic polymorphism discovery at the locus. To probe the impact of *TH* variation on stress-induced disease pathways, we resequenced ~1.2 kbp of 5' promoter, as well as all 13 exons and adjacent intronic regions in 80 ethnically diverse subjects as well as in 422 twins. Twin pairs enabled us to study whether *TH* allelic variation contributed to heritable control of the circulation.

Methods

Subjects

Polymorphism Discovery—A series of 80 unrelated, ethnically diverse individuals was resequenced across the *TH* locus. Their biogeographic ancestries were: 23 European (white), 25 sub-Saharan African (black), 16 East Asian, and 16 Mexican-American (Hispanic). A series of *n* = 213 twin pairs (of several ancestries) was later resequenced at the *TH* promoter.

Human Twin Pairs—*N* = 172 Caucasian twin pairs (119 monozygotic, 53 dizygotic; age 15–84 years) were evaluated for sympathetic traits, including catecholamine production, reflex control of the circulation, and environmental (cold) stress responses.

Hypertension and Population Blood Pressure Extremes—Subjects with hypertension were selected from >50,000 individuals (one-half female and one-half male). In the initial sample, 1266 subjects with the highest (4.9th percentile) and lowest (4.8th percentile) diastolic blood pressures (DBPs) in the population were evaluated; 53% were female.

Experimental Genomics

Genomic DNA was prepared from leukocytes in EDTA-anticoagulated blood, using PureGene extraction columns (Gentra Biosystems, Minneapolis, MN) as described.⁵ Public draft human and mouse genome sequences were obtained from the University of California, Santa Cruz (UCSC) Genome Bioinformatics website and used as scaffolds for primer design and sequence alignment. The base position numbers were from National Center for Biotechnology Information TH (isoform b) source clones NM_000360, NT_009237.17, and NP_000351. Promoter positions were numbered with respect to (–) the TH open reading frame start codon (ATG). PCR primers were designed by Primer3⁶ to span –1155 bp of the proximal promoter, and each of the 13 exons with 50–100 bp of flanking intron. PCR products were treated with exonuclease I and shrimp alkaline phosphatase to remove primers, and then deoxyribonucleotide triphosphate (dNTPs) prior to cycle sequencing with BigDye terminators (Applied Biosystems, Foster City, CA). Sequence was determined on an ABI 3100 automated

sequencer, and analyzed using the Phred/Phrap/Consed suite of software to provide base quality scores, detecting polymorphism and heterozygosity, using PolyPhred⁷ and manually confirmed. A subset of the data was cross-validated manually using base calls from Applied Biosystems software and visual inspection of trace files to identify heterozygotes. Rare single nucleotide polymorphisms (SNPs) were confirmed by re-sequencing in multiple individuals, and from the reverse direction. In addition to initial SNP discovery in a panel of 80 genomic DNA samples, the proximal promoter region of *TH* was resequenced for SNPs scoring in all 172 twin pairs.

Single Nucleotide Polymorphism in Exon 2—The Val81Met polymorphism in *TH* exon 2 (rs6356, A/G) was scored in a two-stage assay.⁸ In 440 individuals ascertained from twin families, genotypic ratios were: A/A, 72; A/G, 197; G/G, 171. Allele frequencies were: Met = A = 39%, Val = G = 61%; Hardy–Weinberg equilibrium $\chi^2 = 1.41$, $P = 0.234$.

Biochemical Traits in Twins: Catecholamines—Plasma and urine catecholamines were measured radioenzymatically.⁹ Urine catecholamine values were normalized to creatinine excretion in the same sample.

Autonomic Phenotyping in Twins

Prolonged Recording of Vital Signs—Blood pressure (in mmHg) and pulse interval (R-R interval or heart period, in msec/beat) were recorded continuously and noninvasively for 5 min in seated subjects with a radial artery applanation device and dedicated sensor hardware (Colin Pilot; Colin Medical Instruments Corp., San Antonio, TX).⁵ Heart rate was recorded continuously with thoracic electrocardiography electrodes to the Colin Pilot. Average, maximum, and minimum values, as well as coefficients of variation were calculated for blood pressure and pulse interval, using the ANS-TDA software (Colin Medical Instruments Corp.).

Environmental Stress Response—Blood pressure and heart rate were recorded continuously and noninvasively with a Colin Pilot during the cold pressor test (CPT; immersion of the left hand in ice water for 60 s, after a 10 min rest).^{5,10,11} We identified at least 3 beats with stable (within $\pm 10\%$) values for blood pressure and heart rate just before and at the end of the CPT.

TH Promoter Strength: Transfection Studies

Human *TH* promoter/reporter plasmids were constructed, sequence-verified, and studied. Promoter positions were numbered upstream (–) or downstream (+) of the start codon (A in ATG as +1 bp). A haplotype-specific promoter fragment (from a wild-type haplotype homozygote), corresponding to *TH* –957 bp/–1 bp, was PCR-amplified from genomic DNA of known homozygotes. The promoter amplicon was subcloned into the promoterless firefly luciferase reporter plasmid pGL3-Basic (Promega, Madison, WI). Only the wild-type *TH* haplotype was amplified from genomic DNA. The other haplotypes were generated by point mutation of the wild-type promoter haplotype-firefly luciferase reporter plasmid (QuikChange mutagenesis; Strata-gene, La Jolla, CA) and resequenced to verify the identity of each allele and haplotype. PC12 pheochromocytoma cells were transfected (at 50–60% confluence, 1 day after 1:4 splitting) with 1 μ g of supercoiled promoter haplotype-firefly luciferase reporter plasmid and 10 ng of the Renilla luciferase expression plasmid pRL-TK (Promega) as an internal control per well, by the liposome method (Superfect; Qiagen, Valencia, CA). The firefly and Renilla luciferase activities in the cell lysates were measured 48 h after transfection, and the results were expressed as the ratio of firefly/Renilla luciferase activity (“Stop & Glo®”; Promega). Each experiment was repeated six times.

Statistical Genetics

Descriptive Statistics—Descriptive statistics (mean, standard error) were computed across all of the twins, using generalized estimating equations (GEEs; PROC GENMOD) in SAS (Statistical Analysis System, Cary, NC).¹²

Heritability in Twins—Heritability (h^2) is the fraction of phenotypic variance accounted for by genetic variance ($h^2 = V_G/V_P$). Estimates of h^2 were obtained by the variance component method implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package (Southwest Foundation for Biomedical Research, San Antonio, TX).¹³

Haplotypes and Linkage Disequilibrium (LD)—Haplotypes were inferred from unphased diploid genotypes with the software package PHASE,¹⁴ assigning the two most likely haplotypes to each diploid individual. We inferred the *TH* promoter haplotypes using 10 SNPs discovered by resequencing 293 unrelated individuals (586 chromosomes) chosen to span four diverse ethnic groups: white (European ancestry), black (sub-Saharan African ancestry), Hispanic (Mexican American), and East Asian.

Association (Marker-on-Trait)—Association studies were performed for the common *TH* promoter alleles (minor allele frequency >5%). Each study subject was categorized according to diploid genotype at a biallelic SNP locus, or carrier status (two, one, or zero copies) of a particular *TH* SNP, haplotype, or diploid haplotype (diplotype). Unpaired *t*-tests evaluated the significance of the *in vitro* haplotype-specific *TH* promoter activity.

Pleiotropy: Bivariate Genetic Analyses—Pleiotropy (genetic covariance for two correlated, heritable traits)¹⁵ was estimated as the parameter RhoG (ρ_G) in SOLAR.¹⁶

Multiple Comparison Adjustments—To adjust for the possibility of multiple comparisons when testing the effect of four *TH* promoter SNPs on autonomic traits, we used the method of Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) proposed by Nyholt¹⁷ and implemented at <http://genepi.qimr.edu.au/general/daleN/SNPSpD/>, to yield an “effective” number of markers within a block of LD.

Permutation Testing—To enable model-free analyses, without reliance on standard asymptotic assumptions, we employed more computationally intensive permutation tests, using the recursive algorithm of Mehta and Patel,¹⁸ as implemented by Clarkson *et al.*¹⁹ Trait values were dichotomized (or converted into two quantiles), to allow construction of 3-by-2 contingency tables (diploid genotype-by-trait).

Results

TH Genomics: Systematic Discovery of Polymorphism across the Locus

Forty-nine SNPs and one tetranucleotide repeat were identified (Fig. 1A). Ten SNPs were located in the ~1 kbp proximal promoter, 14 in coding regions, and 25 in untranslated regions (UTRs) or exon-adjacent intronic regions. Of the 49 SNPs, 13 were common (minor allele frequency >5%), including four SNPs in the proximal promoter, two in coding regions, four in UTRs, and four in introns. Among 14 coding region polymorphisms, 11 specify amino acid substitutions. Val81Met is the most common single nucleotide polymorphisms within complementary DNA sequences (cSNP), with a minor allele frequency at 37.4%; synonymous Lys240Lys is the second most common, at 23.6%. Unusual/rare coding cSNPs (all <1.2%, most at 0.3%) were the balance: Ala6Thr, Arg15His, Ala17Val, Ala23Thr, Arg89Arg, Ala143Thr, Thr225Asn, Arg410Trp, Asp467Asp, Val468Met, Ala492Val, and Gly497Asp.

The tetranucleotide repeat in the first intron had six different alleles, generated by 6–10 repetitions of the core tetranucleotide motif TCAT: (TCAT)₆, (TCAT)₇, (TCAT)₈, (TCAT)₉, and 10-repeat alleles exhibiting two sequence variants: the perfect repeat (TCAT)₁₀, and a (TCAT)₄CAT(TCAT)₅ imperfect repeat, also known as (TCAT)₁₀.

Linkage Disequilibrium (Marker-on-Marker)

Pairwise LD between each common SNP across *TH* was quantified as D' , scaled from 0 → 1. Visual inspection (Fig. 1B) of plots of the LD structure across *TH* reveals two blocks of particularly high LD ($D' > 0.9$): at the 5' (promoter) and mid-to-3' (intron A → exon 12) regions of the gene.

Single Nucleotide Variants in the *TH* Promoter

Ten biallelic variants were discovered (major allele/position upstream of the ATG/minor allele): C-833T, C-824T, G-814A, G-801C, T-741C, A-641G, A-581G, G-494A, C-388T, and G-94T. Alleles at each SNP were in Hardy–Weinberg equilibrium (determined within the largest ethnic group, Caucasian). Four of 10 SNPs (C-824T, G-801C, A-581G, and G-494A) had relatively common minor alleles (>10% frequency); these four occurred within a span of only 331 bp in the proximal promoter.

Twin traits: Heritability

Both plasma catecholamine and urinary catecholamines were significantly heritable. Heritability (h^2) for plasma norepinephrine ($h^2 = 69.9 \pm 4.4\%$, $P < 0.0001$) and urinary epinephrine excretion ($h^2 = 66.7 \pm 5.9\%$, $P < 0.0001$).

Basal blood pressure and heart rate displayed significant heritability, with heart rate substantially more heritable (at $h^2 = 61 \pm 6\%$, $P < 0.0001$) than either systolic blood pressure (SBP) ($h^2 = 26 \pm 8\%$, $P = 0.0016$) or DBP ($h^2 = 18 \pm 9\%$, $P = 0.0359$). Stress-induced changes in vital signs were also heritable, whether expressed as maximal values, absolute changes (maximal minus basal), or percentage changes.

TH Promoter Single Nucleotide Variants: Marker-on-Trait Associations

Univariate Analyses—Significant associations were found for urinary catecholamine excretion (epinephrine and norepinephrine; Fig. 2A), as well as the blood pressure response to environmental (cold) stress (change in SBP and change in DBP; Fig. 2B), with variants at C-824T and A-581G. Variants at G-801C and G-494A did not associate (Fig. 2). The common *TH* coding region/ exon 2 nonsynonymous variant Val81Met did not associate with catecholamines or stress blood pressure changes, nor did the less common (minor allele frequency <5%) coding region variants.

Multivariable Analyses—Since both C-824T and A-581G seemed to influence both catecholamine secretion (Fig. 2A) and the blood pressure response to stress (Fig. 2B), we performed a multivariable analysis of all four common *TH* promoter polymorphisms (C-824T, G-801C, A-581G, and G-494A) in SOLAR in an attempt to discern the most important variant (s). In this analysis, C-824T became the most significant predictor of change in DBP during cold stress ($P = 0.000164$), though G-494A also achieved significance ($P = 0.0276$).

Multiple Comparisons—Since we tested the phenotypic effects of four different promoter SNPs, we re-evaluated the alpha threshold to avoid false positive conclusions. The SNPSpD method of Nyholt¹⁷ tested intermarker correlations within the promoter block of LD to yield an “effective” number of markers at 2.92, indicating that an appropriate alpha threshold to maintain the Type I error rate at ≤5% for a single phenotype would be $P = 0.0171$.

Permutation Tests within the Promoter—Another approach to multiple genotype/phenotype comparisons is the use of model-free exact (permutation) tests. We dichotomized continuous traits about the median value and then constructed 3×2 contingency tables (three diploid genotypes by two trait quantiles). After permutation, the effect of C-824T remained significant upon both urinary norepinephrine excretion ($P = 0.00633$) and the DBP response to cold stress ($P = 0.0157$).

Pleiotropy: Coordinate Effects of Promoter Variant C-824T on Biochemical and Physiological Autonomic Traits

C-824T: Biochemistry and Physiology—C-824T associated with urine catecholamine excretions: epinephrine ($P = 0.0058$, percent variation explained = 5.55%); and norepinephrine ($P = 0.0069$, percent variation explained = 1.52%). The T-824C minor (T) allele associated with higher urinary catecholamine excretion (Fig. 3A) and greater changes of blood pressure response to cold stress (Fig. 3B; Δ SBP: $P = 0.01$, % percent variation = 1.54% and Δ DBP: $P = 0.0069$, percent variation = 3.98%) (Fig. 3A and 3B).

C-824T: Pleiotropy (Norepinephrine and Stress Blood Pressure)—C-824T exerted significant pleiotropic effects on the coupling between blood pressure response to cold stress and urinary norepinephrine (Fig. 3C). Increasing T-824C minor (T) allele copy number increased not only the change in DBP post cold stress, but also urinary norepinephrine excretion (bivariate likelihood ratio analyses: Δ DBP $\chi^2 = 10.4$, $P = 0.0013$; Δ SBP $\chi^2 = 3.91$, $P = 0.048$).

TH Promoter Haplotype Groups

From 10 SNPs in the *TH* proximal promoter, we inferred the presence of 17 haplotypes; the three most common haplotypes accounted for 84.8% of chromosomes examined. The most common haplotype overall (#1, CCGGTAAACG) is rather frequent (>37.1%) in Asian, Hispanic, and white populations, but relatively unusual (15.4%) in black samples. Haplotype #2 (CTGGTAGGCG) is the most common variant in blacks (at 35.6%), though relatively rare in Asians (10%). Similar haplotypes were derived by considering only the four most common promoter SNPs (C-824T, G-801C, A-581G, and G-494A).

Promoter Haplotype Effects on Autonomic Traits

Common Haplotypes—We formed haplotypes from the four most common promoter SNPs (C-824T, G-801C, A-581G, and G-494A), and then tested whether haplotype copy number (0, 1, or 2 per genome) at the diploid locus influenced biochemical or physiological trait means in twins. This approach is feasible (that is, it has sufficient power) for the most common haplotypes in a population. (We have designated these haplotypes #1 [CGAA], #2 [TGGG], and #3 [CCAG].)

The second most frequent promoter haplotype, haplotype #2, displayed copy number-dependent effects on stress change of blood pressure (both Δ SBP: $P = 0.0154$, % variation = 1.90%, and Δ DBP: $P = 0.0004$, % variation = 3.73%) (Fig. 4A), as well as urinary epinephrine ($P = 0.0044$, % variation = 5.7%) and norepinephrine excretion ($P = 0.0125$, % variation = 4.06%). Haplotype #3 displayed associations with both basal heart rate and change of heart rate during cold stress ($P = 0.0104$, % variation = 3.14%). However, there were no associations for trait means with copy number of the most frequent haplotype, haplotype #1.

Diploid Haplotypes (“Diplotypes”)—Haplotype #1/haplotype #2 diploid haplotype pairs influenced change of DBP during cold stress (Δ DBP: $P = 0.0312$, % variation = 3.47%; Fig. 4B). Haplotype #1/haplotype #3 diploid combinations displayed associations with basal HR ($P = 0.0335$, % variation = 4.13%).

Promoter Haplotypes and Pleiotropy: Altering the Coupling between Norepinephrine Release and the Pressor Response to Environmental Stress

The effects of haplotype #2 displayed a pattern of pleiotropy (Fig. 5). Increasing copy number of haplotype #2 augmented changes in DBP ($P = 0.0004$, 3.73% variation explained) during cold stress, as well as urinary norepinephrine excretion ($P = 0.0125$, 4.06% variation explained), and the likelihood ratio test indicated a coordinate effect on the two traits (bivariate $\chi^2 = 14.2$, $P = 0.0002$).

Promoter Variants: Transcriptional Activity Probed by Chromaffin Cell Transfection/Expression

We assayed haplotype-specific gene expression in PC12 chromaffin cells (Fig. 6), with *TH* promoter/luciferase reporters for the two most common promoter haplotypes: #1 (most common) and #2 (second most common). Haplotype #2 was substantially more active in chromaffin cells; Figure 6B illustrates the increased activities of haplotype #2 both *in vitro* (driving transcription) and *in vivo* (determining norepinephrine secretion).

TH Promoter and Human Disease: Hypertension

Figure 7 illustrates a population-based case-control study, in which >1200 subjects were drawn from the highest and lowest 5th percentiles of blood pressure in a primary care practice of >53,000 adults. In a two-way ANOVA, there was a significant sex-by-genotype (*TH* C-824T) interaction on DBP ($P = 0.044$). As expected, sex also influenced DBP ($P < 0.001$). When the ANOVA was run in the presence or absence of C-824T, the results indicated that C-824T variation accounted for 3.4% of population DBP variance. When analyses were separately conducted on the sexes, the C-824T genotype effect was found in males ($P = 0.045$), though not females ($P = 0.985$). Inspection of the bar graph indicates that increasing numbers of the minor (T) allele increased DBP in males, though not females.

To explore the findings in the same group in model-free fashion, without relying on standard asymptotic assumptions, we also employed more computationally intensive permutation tests. We dichotomized subjects by blood pressure status (high versus low), and sex (male versus female). We then conducted 3-by-2 table permutation tests on the effect of C-824T diploid genotype (C/C, C/T, or T/T) on blood pressure status: the effect remained significant in males ($P = 0.023$), though not in females ($P = 0.266$).

Association of (TCAT)_n (Intronic) Alleles with Autonomic Biochemistry and Physiology

At the intronic (TCAT)_n polymorphism, significant associations were found for basal blood pressure and heart rate, post-stress heart rate, and catecholamine secretion. (TCAT)₆ copy number affected basal pulse interval ($P = 0.007$) and heart rate ($P = 0.0003$), as well as post-stress heart rate ($P = 0.0001$). (TCAT)_{10i} copy number affected basal pulse interval ($P = 0.0349$), plasma epinephrine ($P = 0.0133$), and renal norepinephrine excretion ($P = 0.0309$).

Discussion and Conclusions

Summary

We conclude that catecholamine secretory traits are heritable, displaying joint genetic determination (or pleiotropy) (Fig. 5) with autonomic activity and ultimately with blood pressure in the population (Figs. 7 and 8). Interindividual differences in catecholamine secretion are influenced by genetic variation in the adrenergic pathway encoding catecholamine synthesis, especially at the classically rate-limiting step, TH. These results document novel pathophysiological links between a key adrenergic locus, catecholamine metabolism, and

blood pressure (Fig. 8), and suggest new strategies to approach the mechanism, diagnosis, and treatment of systemic hypertension.

Unifying Hypothesis

A schematic formulating our results into a global hypothesis is presented in Figure 8. It outlines the role of intermediate phenotypes and the *TH* candidate gene (especially its C-824T promoter variant) in the determination of hypertension via the initial intermediary of catecholamine metabolism.

Implications for Pathophysiology, Diagnosis, and Treatment

Our results suggest that the adrenergic pathway is centrally involved in the early pathogenesis of hypertension beginning in healthy individuals, perhaps initially by influencing transient blood pressure responses to environmental stress (Figs. 2, 3, and 8). Adrenergic genetic determination of biochemical (Figs. 2A and 3A), physiological (Figs. 2B and 3B), and ultimately disease traits (Fig. 7) suggests that treatments targeting the adrenergic pathway might be beneficial in preventing hypertension, if administered to subjects at specific genetic risk. Our results also suggest the possibility that adrenergic genetic profiling of patients with impaired autonomic activity or increased stress blood pressure might yield practical pharmacogenetic predictors of patients most likely to benefit from sympatholytic therapy.

Future Directions

Our results raise the possibility that profiling subjects for particular adrenergic and signaling polymorphisms would provide an index of risk for or susceptibility to hypertension. This prediction awaits testing in appropriate longitudinal cohorts.

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Figure 1.

Polymorphism discovery at *TH*: the local genomic region. SNP discovery in an ethnically diverse sample: $n = 80$ individuals ($2n = 160$ chromosomes) was studied. Reproduced from *Circulation* (2007; 116(9): 993–1006.) (A) Resequencing strategy. Forty-nine single nucleotide polymorphisms (SNPs) and one tetranucleotide microsatellite were identified. Ten SNPs located in 1 kbp proximal promoter, 14 SNPs located in coding regions, 25 SNPs located in the untranslated regions (UTRs) or in adjacent intronic regions. Of these, 13 SNPs were common (minor allele frequency $\geq 5\%$), including four in the *TH* proximal promoter, two in coding regions, four in the UTRs, and four in intronic regions. Arrows indicate amplicons and the direction of resequencing. (B) Patterns of linkage disequilibrium (LD) across the entire *TH* locus. GOLD (Graphical Overview of Linkage Disequilibrium) plot of point-by-point LD

among 14 polymorphisms with high minor allele frequencies (13.6–42.6%; including the [TCAT]_n in the first intron) spanning 7505 bp at the *TH* locus, and proceeding ~1 kbp upstream (5'; promoter region). The white diagonal is the line of identity ($Y = X$). The Exon/Intron structure of the *TH* locus is shown at top-panel (note the lack of common polymorphisms in the final exon). The blue box at the lower left-hand corner designates the LD block at the proximal promoter. D' , LD parameter (scaled from 0 $\rightarrow$ +1). Results are from $2n = 442$ chromosomes in subjects of European ancestry. (In color in *Annals* online.)

Figure 2.

TH promoter polymorphisms: Region-specific prediction of autonomic traits in twins. Reproduced from *Circulation* (2007; 116(9): 993–1006.) **(A)** Catecholamines: Univariate analyses across the promoter. Significance of association with catecholamine secretion graded by 1/*P*-value. The *P*-value is derived from SOLAR of comparison of individual *TH* promoter SNP in twins. The X-axis indicates the position (in bp upstream of ATG) of the common *TH* promoter SNPs. Significance (horizontal black line): at $P < 0.05$. **(B)** Stress blood pressure: Univariate analyses across the promoter. Significance of association with change of blood pressure response to cold stress graded by 1/*P*-value. The *P*-value is derived from SOLAR of comparison of individual *TH* promoter SNP in twins. The X-axis indicates the position (in bp

upstream of ATG) of the common *TH* promoter SNPs. Significance (horizontal black line): at $P < 0.05$.

Figure 3.

TH promoter polymorphism C-824T: Effects on catecholamine excretion and blood pressure response to stress in twins. Reproduced from *Circulation* (2007; 116(9): 993–1006.) (A)

Influence of *TH* promoter polymorphism C-824T on catecholamine excretion. Urinary epinephrine excretion: SOLAR (adjusted by age, sex) $P = 0.0058$, explaining 5.55% of trait variation. Urinary norepinephrine excretion: SOLAR (adjusted by age, sex) $P = 0.0069$, explaining 1.52% of trait variation. (B) Influence of *TH* promoter polymorphism C-824T on blood pressure response to stress. Change of systolic blood pressure response to cold stress (Δ SBP), $P = 0.01$, explaining 1.54% of the trait variation. Change of diastolic blood pressure response to cold stress (Δ DBP), $P = 0.0069$, explaining 3.98% of the trait variation. (C)

Pleiotropic effects of *TH* promoter polymorphism C-824T on the coupling between diastolic blood pressure response to stress (Δ DBP) and urinary norepinephrine. Bivariate $\chi^2 = 10.4$, $P = 0.0013$.

Figure 4.

TH promoter haplotypes and autonomic traits. Reproduced from *Circulation* (2007; 116(9): 993–1006.) (A) Influence of *TH* promoter haplotype #2 (TGGG) on blood pressure response to stress. Change of systolic blood pressure response to cold stress (Δ SBP), $P = 0.0154$, explaining 1.90% of the trait variation; and diastolic blood pressure response to cold stress (Δ DBP), $P = 0.0004$, explaining 3.73% of the trait variation. (B) Diploid haplotypes: #1 & #2. Influence of *TH* promoter diploid haplotype (haplotypes #1 & #2) on change of diastolic blood pressure response during cold stress, $P = 0.0312$, explaining 3.47% of the trait variation.

Figure 5.

TH promoter haplotypes and pleiotropy. Pleiotropic effect of *TH* promoter haplotype #2 on the coupling between change of diastolic blood pressure during cold stress (ΔDBP) and renal norepinephrine excretion. Bivariate $\chi^2=14.2$, $P=0.0002$. Reproduced from *Circulation* (2007; 116(9): 993–1006.)

Figure 6.

Function of *TH* promoter haplotypes: Coordinate effects on *in vitro* *TH* transcription as well as *in vivo* catecholamine production. Results from promoter haplotype reporter transfection into PC12 chromaffin cells *in vitro* are shown on the X-axis, while renal norepinephrine excretion *in vivo* results for haplotype homozygotes are shown on the Y-axis. Haplotype #1 (CGAA) displays weaker promoter strength *in vitro*, while CGAA/CGAA homozygotes had diminished renal norepinephrine secretion *in vivo*. Conversely, haplotype #2 (TGGG) showed increased activity both *in vitro* and *in vivo*. Reproduced from *Circulation* (2007; 116(9): 993–1006.)

Figure 7.

TH promoter polymorphism and hypertension: Population blood pressure extremes. C-824T genotype interacts with sex to determine blood pressure in the population. The sample constituted >1200 individuals selected from the highest and lowest 4th–5th percentiles of DBP in a primary care population of >53,000. By two-way ANOVA there was a significant genotype-by-sex interaction ($F = 3.14$, $P = 0.044$), with results most prominent in males. Males alone also had a significant effect of genotype on DBP ($F = 3.12$, $P = 0.045$). Reproduced from *Circulation* (2007; 116(9): 993–1006.)

Figure 8.

Intermediate phenotypes. The “intermediate phenotype” viewpoint formulates a series of time-dependent phenotypic manifestations of complex trait genetic variants: biochemical traits are hypothesized to be determined directly by genotype, followed later by physiological traits, and ultimately by late-penetrance disease (such as hypertension). Here the concept is illustrated by the findings at *TH* in the current study: the *TH* promoter variant C-824T initially alters norepinephrine production, subsequently influencing stress blood pressure responses (gene-by-environment interaction), finally predisposing to the development of fixed/established hypertension in the population. Reproduced from *Circulation* (2007).