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REVIEW PAPER

Human Tyrosine Hydroxylase Natural Allelic Variation: Influence on Autonomic Function and Hypertension

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Abstract The catecholamine biosynthetic pathway consists of several enzymatic steps in series, beginning with the amino acids phenylalanine and tyrosine, and eventuating in the catecholamines norepinephrine (noradrenaline) and epinephrine (adrenaline). Since the enzyme tyrosine hydroxylase (TH; tyrosine 3-mono-oxygenase; EC 1.14.16.2; chromosome 11p15.5) is generally considered to be rate-limiting in this pathway, probed as to whether common genetic variation at the *TH* gene occurred, and whether such variants contributed to inter-individual alterations in autonomic function, either biochemical or physiological. We began with sequencing a tetranucleotide (TCAT) repeat in the first intron, and found that the two most common versions, $(TCAT)_6$ and $(TCAT)_{10i}$, predicted heritable autonomic traits in twin pairs. We then conducted systematic polymorphism discovery across the ~8 kbp locus, and discovered numerous variants, principally non-coding. The proximal promoter block contained four common variants, and its haplotypes and SNPs (especially C-824T, rs10770141) predicted catecholamine secretion, environmental stress-induced BP increments, and hypertension. Finally, we found that two of the common promoter variants, C-824T (rs10770141) and A-581G (rs10770140), were functional in that they differentially affected transcriptional activity of the isolated promoter, disrupted recognition motifs for specific transcription factor binding, altered the promoter responses to the co-transfected (exogenous) factors, and bound the endogenous factors in the chromatin fraction of the nucleus. We concluded that common variation in the proximal *TH* promoter is functional, giving rise to changes in autonomic function and consequently cardiovascular risk.

Keywords Catecholamine · Stress · Chromaffin · Catecholamine

Abbreviations

PC12	Rat pheochromocytoma cell line
TH	Tyrosine hydroxylase (tyrosine 3-mono-oxygenase;EC 1.14.16.2)
TCAT	Tetranucleotide repeat in *TH* intron-A

Introduction

Tyrosine hydroxylase (*TH*; tyrosine 3-mono-oxygenase; EC 1.14.16.2; chromosome 11p15.5), which catalyzes the formation of DOPA from dopamine, is generally considered to be the rate-limiting enzyme in the catecholamine biosynthetic pathway (Flatmark and Stevens 1999). The human *TH* locus, on chromosome 11p15 (Chitbangonsyn et al. 2003), contains 13 exons/12 introns spanning approximately 8 kbp. Although a single-copy gene, *TH* produces four different

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types of mRNA through alternative splicing of a single primary transcript (Grima et al. 1987), indicating one novel level of regulating catecholamines. Substantial loss of *TH* enzymatic activity has profound consequences both in humans (Flatmark and Stevens 1999) and in mice with targeted ablation of the *TH* locus (Zhou et al. 1995).

TH Microsatellite Polymorphism and Hypertension

Essential (idiopathic) hypertension develops over several decades of life. The human *TH* locus bears several examples of common natural allelic variation, such as the tetranucleotide repeat [or microsatellite polymorphism, $(TCAT)_n$] in its first intron, which associate with essential hypertension (Sharma et al., 1998). The *TH* tetranucleotide repeat $(TCAT)_n$ occurs in 5–11 head-to-tail copies. Barbeau et al. (2003) found that $(TCAT)_6$ and $(TCAT)_{10i}$ alleles seemed to be cardioprotective by association with attenuation of the hemodynamic response to stress with increasing age, while $(TCAT)_7$ seemed to be deleterious by their association with higher resting systolic blood pressure and greater hemodynamic response to stress with increasing body mass index. Sharma et al. (1998) found that the $(TCAT)_{10}$ ("E") allele was over-represented in hypertensive subjects, whereas the $(TCAT)_9$ ("D") allele frequency was increased in control (normotensive) subjects. Wei et al. (1997) found $(TCAT)_9$ to be associated with higher norepinephrine levels.

In order to probe the role of *TH* polymorphism in stress-induced disease pathways, we used a classical human twin study design (Boomsma et al. 2002). Exploring the effects of particular $(TCAT)_n$ alleles on "intermediate phenotypes," we stratified each individual on the basis of number of copies of the allele. We found that the two most common alleles, $(TCAT)_6$ and $(TCAT)_{10i}$, influenced a number of autonomic traits, both biochemical and physiological: $(TCAT)_6$ copy number affected basal pulse interval and heart rate, and post-stress heart rate. $(TCAT)_{10i}$ copy number affected basal pulse interval, plasma epinephrine, and renal norepinephrine excretion (Zhang et al. 2004). We documented the heritability of blood pressure, and found that $(TCAT)_6$ allele frequencies differed among individuals stratified by genetic risk (family history) of hypertension: in particular, family history-positive individuals were less likely to bear the $(TCAT)_6$ allele. Since increasing $(TCAT)_6$ allele copy number was associated with lower basal and stress-induced heart rates, the results obtained suggested a mechanism whereby $(TCAT)_6$ alleles may be protective against the future development of hypertension.

The $(TCAT)_n$ motif can bind such transcription factors as AP1[10] or ZNF191 (Albanese et al. 2001) and may function as a transcriptional enhancer when tested in transfected/expressed promoter/reporter plasmids. However, in transfected *TH* promoter/intron/reporter studies (Albanese et al. 2001), the $(TCAT)_n$ repeat *silences* transcription in a copy number-dependent way; by contrast, in vivo we observed directionally opposite associations of common $(TCAT)_n$ alleles with autonomic function: $(TCAT)_{10i}$ with activation and $(TCAT)_6$ with diminution of sympathetic outflow (Zhang et al. 2004). Thus, transcriptional effects of the $(TCAT)_n$ could *not* mechanistically explain common variation in human autonomic function. Therefore, we carried out systematic polymorphism discovery at the locus.

Discovery of Common *TH* Variants Governing Transcription, Human Autonomic Activity and Blood Pressure In Vivo

In order to probe the possible underlying impact of *TH* variation on stress-induced disease pathways, we re-sequenced $\sim$1.2 kbp of 5′ promoter as well as all 13 exons and adjacent intronic regions for rare and common variants in 80 ethnically diverse subjects as well as 422 twins. Plasma and urine catecholamines were measured by radioenzymatic assay (Kennedy and Ziegler 1990).

In the coding region, we found two common bi-allelic variants, only one of which was non-synonymous: Val81-Met (at 37.4%) which did not associate with the autonomic traits we studied. We also found 12 unusual coding region variants, but the non-synonymous variants had minor allele frequencies of only 0.3–0.6%, not sufficient to account for the common population associations.

Thus, we turned to potential regulatory (non-coding) variants and found 10 SNPs in the proximal promoter region, four of which (C-824T, G-801C, A-581G, and G-494A) were common (minor allele frequencies >10%). Statistically, C-824T (rs10770141) and A-581G (rs10770140) seemed to influence both catecholamine secretion and the blood pressure response to environmental stress. A multivariable analysis further indicated that C-824T became the most significant predictor of change in DBP during cold stress. The -824T allele is associated with increased catecholamine production, increased blood pressure increments in response to stress, and extreme blood pressure values in the population. It was tempting to speculate that the functional variation that we observed in -824T carriers could be the outcome of environmental selective pressures on alleles augmenting catecholaminergic function (Rao et al. 2007).

Mechanistic Studies of Human *TH* Promoter Genetic Variation

More recently, we performed additional studies to test whether such *TH* promoter variants were by themselves

functional (Zhang et al. 2010). First, common haplotypes (at least 1–5% frequency) of the *TH* promoter were generated by site-directed mutagenesis, then verified by sequencing, and inserted into luciferase reporter vectors. After transfection into PC12 cells, such human *TH* promoter haplotypes showed substantial differences in luciferase reporter activity. By two-way ANOVA on luciferase activity, two of the four common variants (C-824T [rs10770141] and A-581G [rs10770140]) altered transcriptional activity. Comparison of these *in cells* results with the effects of the same 4 variants in vivo indicates that the same variants (C-824T and A-581G) that exert the greatest effect on *TH* transcription *in cells* also have the most pronounced effect on human catecholamine secretion in vivo. In addition, the -824T allele displayed an augmented response to typical chromaffin cell secretory stimuli, such as nicotine or PACAP. Under basal circumstances, greater activity of the T allele was observed, and when stimulated by nicotine, C-824T responded differentially, with an increased effect of the drug on the T allele; when stimulated by PACAP, the increased response of the T allele was even more apparent. The -581G allele also responded differentially to PACAP. In the basal state, greater activity of the G allele was apparent; during nicotine stimulation, greater activity of the G allele was noted, as was the case during response to PACAP. We further probed the functional significance of C-824T and A-581G by co-transfection of sequence-predicted *trans*-activating factors *in cells*: MEF2, SRY, and FOXD1 differentially activated C-824T, whereas the G/C-rich binding factors SP1, AP2, and EGR1 differentially activated A-581G. At C-824T, the co-transfected human MEF2 increased *TH* promoter expression to 131.3% on the T allele, but had little effect on the C allele. The MEF2 dominant-negative mutant mMEF2 decreased *TH* promoter expression down to 71.3% on the T allele, but had little effect on the C allele. Thus, at C-824T, factor MEF2 acted in a directionally coordinate fashion (at $T > C$) to explain the in vivo trait associations, whereas at A-581G, factors SP1, AP2, and EGR1 displayed similar differential actions (at $G > A$). Chromatin immunoprecipitation (ChIP) confirmed the interaction of the endogenous transcription factors with these motifs in the nucleus (Zhang et al. 2010).

Conclusions

We conclude that inter-individual variability in catecholamine secretion is controlled in substantial part by genetic variation in the adrenergic pathway encoding cate cholamine synthesis, especially at the classical rate-limiting step, *TH*. *TH* promoter common polymorphisms C-824T and A-581G are not only statistical predictors of catecholamine secretory and stress BP response traits in vivo, but also causally responsible for alterations in transcriptional efficiency of the *TH* gene. Our results thus document 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 autonomic dysfunction as well as systemic hypertension.

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