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A functional intronic variant in the tyrosine hydroxylase (TH) gene confers risk of essential hypertension in the Northern Chinese Han population

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ABSTRACT

The TH (tyrosine hydroxylase) gene encodes the rate-limiting enzyme of catecholamine biosynthesis, and is involved in the pathogenesis of hypertension, but the relationship of its variants with hypertension has not been extensively studied. We designed a case-controlled study consisting of 503 HT (hypertensive) individuals and 490 NT (normotensive) individuals matched by region, age and gender to systematically investigate the association between the TH gene and hypertension. Based on the HapMap and dbSNP (where SNP is single nucleotide polymorphism) data, four SNPs, rs6356 A > G, rs6357 G > A, rs2070762 T > C and rs1800033 A > G in the TH gene were selected for genotyping. Rs1800033 was not polymorphic in our study population. No significant differences were observed for distributions of rs6356 and rs6357 between the HT and NT groups. However, both the genotype and allele frequencies of rs2070762 showed significant differences between cases and controls ($P < 0.001$ and $P = 0.005$ respectively). In haplotype analysis, a total of eight haplotypes were observed in the entire population and the overall frequency distributions differed significantly between the HT and NT groups. Specifically, haplotype A-A-C (rs6356-rs6357-rs2070762) occurred only in the HT group and A-G-C occurred more commonly in HT subjects than in NT subjects ($P = 0.003$ and $P = 0.013$ respectively). Compared with the most common haplotype A-G-T, the adjusted OR (odds ratio) was 1.83 [95% CI (confidence interval), 1.20–2.79; $P = 0.0049$] for haplotype G-G-C and 20 ($P < 0.0001$) for the haplotype A-A-C. Functional analysis showed that the C allele of rs2070762 functioned as an enhancer in the absence of binding by unidentified transcriptional repressor(s). These results provide evidence for an association of the functional intronic rs2070762 with essential hypertension.

Key words: association study, essential hypertension, haplotype, transcriptional repressor, tyrosine hydroxylase.

Abbreviations: BMI, body mass index; BP, blood pressure; CHB, Han Chinese in Beijing; CI, confidence interval; DBP, diastolic BP; EMSA, electrophoretic mobility-shift assay; HDL-C, high-density lipoprotein-cholesterol; HT, hypertensive; LD, linkage disequilibrium; NT, normotensive; OR, odds ratio; SBP, systolic BP; SNP, single nucleotide polymorphism; TH, tyrosine hydroxylase.

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INTRODUCTION

Essential hypertension is the most common risk factor for cardiovascular, cerebrovascular and renal diseases [1], affecting up to 30% of the adults in Western societies [2] and 27.2% of the adults aged 35–74 years in China [3]. Prevailing evidence has shown that essential hypertension is a complex disease where both multiple minor genetic and environmental factors interact [4], and the genetic contribution to BP (blood pressure) levels ranges from 30 to 60% [5]. One approach to identify genetic factors associated with essential hypertension is candidate gene strategies, in which specific candidates have been tested for linkage and association with BP or the diagnosis of hypertension to identify hypertension-predisposition genetic loci. Fine clarification of hypertension-predisposition genetic loci in the aetiology of hypertension may contribute to the identification of individuals at high risk and the development of more efficient therapeutic strategies for the disease[6].

TH (tyrosine hydroxylase) is the first and rate-limiting enzyme in the synthesis of catecholamines, including dopamine, noradrenaline and adrenaline, by converting tyrosine into L-dopa [7], and plays important roles in the regulation of the central and sympathetic nervous system and cardiovascular function [8]. Abnormal catecholamine transmission is implicated in essential hypertension [9], plasma catecholamine is observed to be increased in the development of hypertension and noradrenaline is pivotal in the regulation of BP [8]. These results indicate that the TH gene plays an important role in the pathogenesis of hypertension in humans, therefore the TH gene is considered as a candidate for involvement in genetic susceptibility of the aetiology of hypertension.

A single copy human TH gene is located on chromosome 11 p15.5 and consists of 13 primary exons [10]. Several variants within this gene have been identified and used in a number of studies testing the association of the TH gene with nervous system diseases such as affective disorders [11–13], schizophrenia [6,14–17] and nicotine dependence [18–20]. However, only a few studies have been conducted to test the relationship between the TH gene and essential hypertension. Among these studies controversial conclusions have emerged. Sharma et al. [9] reported a strong association of the TH01 microsatellite, a tetranucleotide repeat in intron 1 of the TH gene, and essential hypertension in a case-controlled study. Also, TH01 was considered to exert a powerful, heritable effect on autonomic control of the circulation and thus might have implications in later development of cardiovascular disease traits such as hypertension by a twin study design [21]. Nevertheless, an association study of TH01 and myocardial hypertrophy and death from myocardial infarction suggested that TH01 was not associated with a significant increase in the incidence of myocardial hypertrophy or other hypertension-associated diseases [22]. These studies only focused on the investigation of the association of the TH01 variant with hypertension, but other polymorphisms in the TH gene have not been investigated.

Our previous report [23] found that rs2070762 of the TH gene was independently associated with essential hypertension in Chinese subjects. In the present study, we systematically analysed the association of SNPs (single nucleotide polymorphisms) and haplotypes of the TH gene with hypertension using a well-matched case-controlled design. Furthermore, the biological relevance of the significantly associated rs2070762 in intron 12 of the TH gene was studied by various functional assays *in vitro*.

MATERIALS AND METHODS

Study population

The local bioethical committee approved the study protocol and informed consent for participation was obtained from all subjects. All DNA samples and clinical data were obtained from the previous International Collaborative Study of Cardiovascular Disease in Asia (InterASIA) [3]. InterASIA stratified China into north and south, as delineated by the Yangtze River. The sample population involved in the present investigation consisted of 993 [503 HT (hypertensive) patients and 490 age-, gender- and area-matched NT (normotensive) controls] unrelated Northern Han Chinese. All measurements and interviews were taken under standard conditions as previously described [3]. All HT patients were defined as having an average SBP (systolic BP) $\geqslant$ 160 mmHg and/or an average DBP (diastolic BP) $\geqslant$ 100 mmHg. The control subjects had a SBP $<$ 140 mmHg and a DBP $<$ 90 mmHg. Blood pressure values of HT patients receiving treatment were adjusted according to the algorithm described in analyses of Framingham data [24]. Overall, 16.7% of hypertension cases were ascertained based on current treatment for hypertension and the above criteria. None of the subjects had secondary hypertension, coronary heart disease or diabetes.

SNP selection and genotyping

We searched HapMap data (http://www.hapmap.org/), and six SNPs have been successfully genotyped in Han Chinese in Beijing (termed CHB). Three of the six SNPs available from the HapMap were polymorphic in the CHB population and only two of them (SNP rs6356 and rs2070762) had the minor allele frequencies over 0.05. Rs6356 results in a valine to methionine residue change at codon 81, and rs2070762 is located within intron 12. These two SNPs were in weak LD (linkage disequilibrium; $D' = 0.703$ and $r^2 = 0.074$ based on the HapMap data), so we selected both of them for genotyping. In addition, another two exonic SNPs, rs6357 and rs1800033

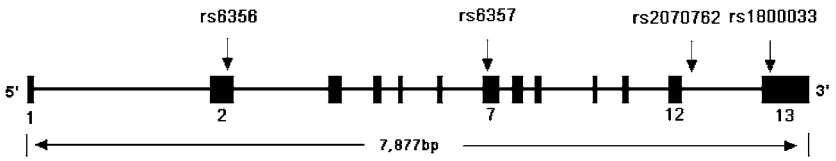

Figure 1 The genomic structure of the human TH gene and the relative physical positions of the four selected SNPs
The exons are indicated by black boxes, and the relative physical positions of the four SNPs chosen to investigate in the present study are indicated by arrows.

in the dbSNP database, were examined in the present study. Rs6357 is synonymous at codon 240, and rs1800033 is non-synonymous resulting in a methionine to valine residue change at codon 468. The genomic structure of the TH gene and the relative physical positions of the four SNPs we chose for the present study are shown in Figure 1.

The four SNPs, rs6356, rs6357, rs2070762 and rs1800033, were genotyped in all 993 subjects by PCR/RFLP (restriction fragment length polymorphism) protocols. The primers and enzymes used for genotyping of rs6356, rs6357 and rs2070762 have been described previously [18], and the primers used for genotyping of rs1800033 were 5′-GACTCTGCCCGCCAGTTGA-3′ (sense) and 5′-GAGCCTCTGGAGCTGCTTGG-3′ (antisense). Amplification conditions for this SNP involved an initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C, 69 °C and 72 °C for 15, 15 and 30 s respectively, and a final step at 72 °C for 2 min. The PCR product was then digested using enzyme NlaIII.

Construction of the luciferase reporter gene

The genomic sequence of the TH gene containing the site of rs2070762 was amplified by PCR from one individual homozygous for the C allele and one individual homozygous for the T allele. The PCR primers were tailored to incorporate a BamHI site at the 5′-end and a SalI site at the 3′-end of the amplified fragments. These 410-bp genomic fragments, spanning between 2142706 and 2143115 on chromosome 11 p15.5, were then purified, digested with the two designated restriction endonucleases, and further subcloned into the BamHI and SalI sites of the firefly-luciferase-expressing pGL3-promoter vector (Promega) to create two plasmids: pGL3-promoter-C and pGL3-promoter-T. Both constructs were sequenced to verify that the only ambiguity was the polymorphic site.

Cell cultures, transfections and luciferase assays

SH-SY5Y cells (human neuroblastoma) were grown in RPMI-1640 medium with 10 % FBS (fetal bovine serum), 5 % heat-inactivated donor horse serum and 10 μg/ml penicillin/streptomycin at 37 °C and 5 % CO_2. Cells were seeded at a density of 2.5×10^5 cells per well in a 12-well plate at 24 h prior to transfection in medium without antibiotics. Transfections were performed with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's protocol. Approx. 2 μg of the luciferase reporter gene was co-transfected with 0.5 μg of pRL-TK (*Renilla* luciferase; Promega) as an internal control for variations in transfection efficiency. Transfection using pGL3 promoter vector without an insert was used as a negative control. The transfected cells were harvested after 24 h, and the luciferase activity was measured using the dual-luciferase reporter assay system (Promega) using a luminometer (TD-20; Turner Designs).

Preparation of nuclear extracts

Nuclear extracts from SH-SY5Y cells were prepared with NE-PER nuclear and cytoplasmic extraction reagents kit (Pierce) according to the manufacturer's protocol. Protein concentrations were determined using a BCA (bicinchoninic acid) assay (Pierce), and the nuclear extracts were stored at − 80 °C.

EMSA (electrophoretic mobility-shift assay)

Synthetic double-stranded oligonucleotide probes (C: ACCGACCCCTGG**C**TGCAGCAGCCCC and T: ACCGACCCCTGG**T**TGCAGCAGCCCC) corresponding to the genomic sequence in intron 12 of the TH gene with either a C or T allele at the rs2070762 site (shown in bold type) were labelled with biotin (Shanghai Sangon Biological Engineering Technology and Services). EMSAs were performed by using the LightShift™ chemiluminescent EMSA kit (Pierce). For each gel-shift reaction (20 μl), a total of 20 fmol of biotin-labelled probes were combined with 15 μg of nuclear extract prepared from SH-SY5Y cells, 1 μg of poly (dI-dC), and 1 × binding buffer (10 mM Tris/HCl, 50 mM KCl and 1 mM dithiothreitol, pH 7.5). For competition assays, a 200-fold molar excess of unlabelled C or T probe was pre-incubated for 5 min at room temperature (25 °C) with nuclear extracts before the addition of the labelled probe. The reaction mixture was resolved on a non-denaturing 6 % acrylamide gel in 0.5 × TBE [Tris/borate/EDTA (1 × TBE = 45 mM Tris/borate and 1 mM EDTA)] buffer, and the electrophoresized binding reactions were then

Table 1 Characteristics of the HT and NT groups

Values are means ± S.D. LDL-C, low-density lipoprotein cholesterol; Drinkers, the number of alcohol consumers who drank not less than 12 times during the year ahead of the interview; Smokers, the number of cigarette consumers who had smoked not less than 100 cigarettes.

Characteristics	HT group	NT group	*P* value
n	503	490	
Gender (male/female)	262/241	257/233	0.909
Age (years)	53.6 ± 9.3	53.5 ± 9.2	0.921
BMI (kg/m^2)	26.3 ± 3.85	24.30 ± 3.56	< 0.001
SBP (mmHg)	177.1 ± 28.0	117.5 ± 11.6	< 0.001
DBP (mmHg)	104.3 ± 12.3	75.0 ± 8.0	< 0.001
Cholesterol (mmol/l)	5.22 ± 0.98	5.06 ± 1.05	0.012
Triacylglycerol (mmol/l)	1.70 ± 1.06	1.43 ± 0.86	< 0.001
Creatinine (μmol/l)	70.8 ± 15.2	68.9 ± 12.1	0.028
HDL-C (mmol/l)	1.25 ± 0.30	1.32 ± 0.34	0.001
Glucose (mmol/l)	5.93 ± 1.79	5.59 ± 1.68	0.003
LDL-C (mmol/l)	3.19 ± 0.85	3.09 ± 0.87	0.054
Smoking (%)	40.6 (204)	43.1 (211)	0.424
Drinking (%)	34.4 (173)	33.5 (164)	0.758

transferred on to nylon membrane, and cross-linking was performed for 5 min with a UV cross-linker. The signal of the biotin-labelled DNA bound to the membrane was detected with a LightShift™ chemiluminescent EMSA kit (Pierce) according to the manufacturer's protocol.

Statistical analysis

The data were analysed using SAS statistical software (SAS Institute) and the program R (http://www.r-project.org). Quantitative data were expressed as means ± S.D. The differences in clinical characteristics between the HT and NT groups were assessed using the *t* test for quantitative variables and χ^2 test for categorical ones. The frequencies of the alleles and genotypes between the HT and NT groups were compared using the χ^2 test. Pairwise LD coefficients, r^2 and D' were calculated using the GOLD program [25]. Haplotype evaluation across identified SNPs, including haplotype evaluation and comparison and regression, were performed using the package Haplo.stats [26]. In regression analysis ORs (odds ratios) and 95 % CIs (confidence intervals) were computed from generalized linear regression parameters derived from haplo.glm. Luciferase assay data were assessed using the Student's *t* test using SigmaPlot (SPSS) and a *P* value less than 0.05 was considered statistically significant.

RESULTS

Clinical characteristic and SNP analysis

The clinical characteristics of all subjects are shown in Table 1. Among these cardiovascular risk factors, BMI (body mass index), total cholesterol, triacylglycerols, creatinine and HDL-C (high-density lipoprotein cholesterol) differed significantly between the HT and NT groups. Three out of four SNPs were identified in the present population, leaving rs1800033 non-polymorphic in either the HT or NT group (Table 2). The frequencies of the genotypes of rs6356 in the HT group were similar to those in the NT group ($P = 0.596$). Also no association of rs6357 with hypertension was found ($P = 0.756$ for the comparison of frequencies of genotypes). The frequency of the C allele of rs2070762 in the HT group was higher than in the NT group (0.48 compared with 0.42), and a significant association with hypertension was observed ($P < 0.001$). Except for rs2070762, rs6356 and rs6357 were in HWE (Hardy–Weinberg equilibrium) in both the HT and NT groups. We retyped rs2070762 in 98 randomly selected individuals and found 100 % concordance with our original genotyping result, indicating that the genotyping error could be excluded.

Haplotype analysis

The rs6357 and rs2070762 polymorphisms were found to be in LD (Table 3), but the correlation between them is somewhat weak, as denoted by low r^2 values of 0.015 in the HT group and 0.004 in the NT group. A total of eight possible haplotypes were observed based on the genotypes of the three SNPs in the entire subject sample, and only four haplotypes had the frequencies over 0.05 (Table 4). Comparison of the distributions of overall haplotypes demonstrated a significant difference between the HT and NT groups ($P < 0.0001$). This difference

Table 2 Genotype and allele distributions of SNPs in the TH gene in the HT and NT groups

P values are for the comparision between the HT and NT groups. *Probability was computed by Fisher's exact test. †Genotype was missing for three patients and five control subjects. ‡Genotype was missing for one control subject.

SNPs	HT group ($n = 503$)	NT group ($n = 490$)	*P*
rs6356†	62.2 % AA, 32.6 % AG, 5.2 % GG	62.1 % AA, 34.0 % AG, 3.9 % GG	0.596
	78.5 % A, 21.5 % G	79.1 % A, 20.9 % G	0.756
rs6357	85.7 % GG, 13.9 % GA, 0.4 % AA	86.9 % GG, 12.9 % GA, 0.2 % AA	0.756*
	92.6 % G, 7.4 % A	93.4 % G, 6.6 % A	0.528
rs2070762‡	11.5 % TT, 80.1 % TC, 8.4 % CC	26.0 % TT, 63.8 % TC, 10.2 % CC	< 0.001
	51.6 % T, 48.4 % C	57.9 % T, 42.1 % C	0.005

Table 3 Test of LD between the three SNPs

Group	r^2	D'	P
HT group			
rs6356-rs6357	0.042	0.379	< 0.001
rs6357-rs2070762	0.015	0.449	< 0.001
rs6356-rs2070762	0.027	0.306	< 0.001
NT group			
rs6356-rs6357	0.114	0.651	< 0.001
rs6357-rs2070762	0.004	0.289	0.0395
rs6356-rs2070762	0.020	0.235	< 0.001

was especially remarkable for the haplotype A-A-C (in the order of rs6356-rs6357-rs2070762) only observed in the HT group ($P = 0.0026$). Moreover, haplotype A-G-C was significantly overrepresented in the HT group ($P = 0.0129$), and haplotype A-G-T was over-represented in the NT group ($P = 0.0008$). Compared with the most common haplotype A-G-T, the adjusted OR was 1.83 (95 % CI, 1.20–2.79; $P = 0.0049$) for haplotype G-G-C and 20 ($P < 0.0001$) for the haplotype A-A-C (Table 4).

Allele-specific effect of the rs2070762 on transcriptional activity

Considering that transcriptional regulatory elements might be located in the intron region, we performed biological assays to investigate whether the rs2070762 had a direct effect on promoter transcriptional activity. A genomic fragment of the TH gene carrying either the C or T allele was inserted into the BamHI and SalI sites of the firefly-luciferase-expressing pGL3-promoter vector (Figure 2A). The activity of these luciferase reporter gene constructs was assessed in transient transfection assays in SH-SY5Y cells. The luciferase activity of the variant C construct was 3- to 4-fold higher than that of the common T construct or empty vector ($P < 0.005$; Figure 2B). This result clearly indicated that the C allele drastically increased the expression of the reporter gene driven by a SV40 promoter, demonstrating that the allelic change from T (non-susceptible allele) to C (susceptible allele) in rs2070762 might be a functional enhancer element regulating gene expression.

Allele-specific effect of the rs2070762 on binding of nuclear proteins to intron 12 of the TH gene

To elucidate specific nuclear factors that might bind a disease-susceptible allele, we analysed the sequence around the rs2070762 site using TRANSFAC software, but no known protein was predicted to bind to this DNA segment. Despite this, we performed EMSAs to determine the nuclear factors that might bind to oligonucleotides corresponding to the genomic sequence of the C or T allele of rs2070762, and determined whether the binding of the transcription factor(s) differed for the C and T alleles. Using nuclear extracts from the SH-SY5Y cells and biotin-labelled probes corresponding to the C or T allele (Figure 3A), we observed a band in the T allele but not in the C allele (Figure 3B, lane 5), and this shifted band could be completely abolished by 200-fold unlabelled T allele probes (Figure 3B, lane 6), indicating specific binding of nuclear protein(s) to the oligonucleotide corresponding to the genomic sequence of the T allele.

DISCUSSION

Essential hypertension is a complex disease influenced by numerous genetic and environmental factors and their interactions. We sought to identify genetic factor(s) that might confer individual susceptibility to essential hypertension. We analysed rs6356, rs6357, rs2070762 and rs1800033 of the TH gene in 503 HT subjects and 490 NT subjects, and found that the C allele of rs2070762 and two haplotypes consisting of this allele were significantly associated with the increased risk of essential hypertension. The SNP rs6356 did not show any association with hypertension, although it has potential function by

Table 4 Association of the TH gene haplotypes with essential hypertension

*Loci are arranged in the order rs6356, rs6357, rs2070762. †Covariates were adjusted.

Haplotypes*			Frequencies					
			All	HT	NT	P value	OR (95 % CI)	P value†
A	A	C	0.008	0.012	0.000	0.003	> 20.00	< 0.0001
A	A	T	0.020	0.025	0.019	0.110	1.15 (0.36–3.71)	0.813
A	G	C	0.319	0.337	0.304	0.013	1.36 (0.99–1.87)	0.056
A	G	T	0.441	0.412	0.466	0.001	Reference	–
G	A	C	0.016	0.008	0.022	0.537	0.91 (0.21–3.98)	0.895
G	A	T	0.027	0.029	0.026	0.482	1.01 (0.41–2.48)	0.987
G	G	C	0.111	0.128	0.095	0.082	1.83 (1.20–2.79)	0.005
G	G	T	0.060	0.050	0.068	0.234	0.87 (0.44–1.69)	0.673

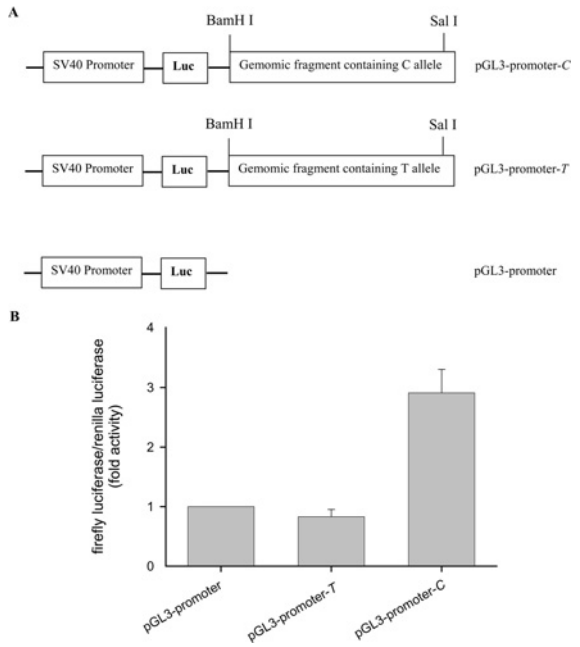

Figure 2 Allelic change from T (non-susceptible allele) to C (susceptible allele) in rs2070762 enhanced transcriptional activity of a heterologous promoter in SH-SY5Y cells

(A) Schematic of the plasmid constructs. Genomic fragment of intron 12 of the TH gene carrying either the C or T allele at the rs2070762 site was cloned downstream of the firefly-luciferase-expressing pGL3-promoter vector into the BamHI and SalI sites. (B) SH-SY5Y cells were co-transfected with individual firefly-luciferase-expressing plasmid (pGL3-promoter, pGL3-promoter-C, pGL3-promoter-T) and pRL-TK as described in the Materials and methods section. Cells were harvested after 24 h, and relative luciferase activity was determined. Luciferase activity of the variant C construct was 3- to 4-fold higher than that of the common T construct and control vector (pGL3-promoter) ($P < 0.005$). Values are present as the fold-induction compared with the promoter vector without insert. Values are means $\pm$ S.E.M. of three different experiments, each performed in triplicate.

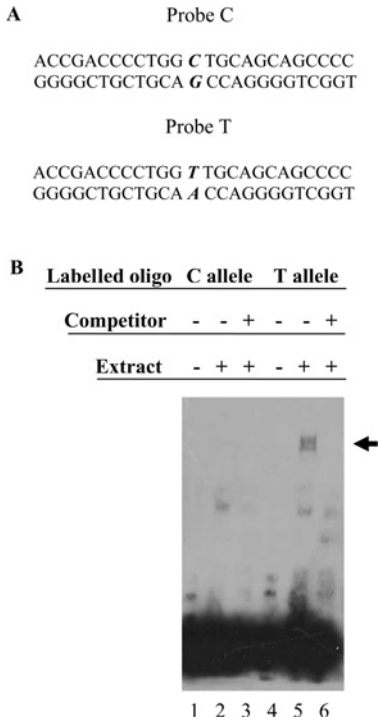

Figure 3 The C and T alleles of rs2060762 in intron 12 of the TH gene differentially bound the transcription factor complex

(A) The double-stranded oligonucleotide probes used in EMSA assays. The probes differ only at the rs2060762 site (# bold, italic). (B) EMSA experiment showing specific binding of unknown nuclear factor(s) to the T allele. EMSA was performed using biotin-labelled probe C or probe T with SH-SY5Y cell nuclear extract, with or without competition from unlabelled probe C or probe T. The arrow indicates the band showing specific binding of nuclear factors to the T allele. The experiments were repeated three times with similar results.

resulting in a change from a valine to methionine residue at codon 81 within exon 2 of the TH gene. This negative result was consistent with a previous study which involved 73 young borderline HT subjects [9]. We have found the absence of haplotype A-A-C in the NT group, probably owing to the relative low frequency of the A allele of rs6357 (0.074 in the HT group and 0.067 in the NT group respectively). Haplotype G-G-C was associated with the risk of essential hypertension after adjustment of cardiovascular risk factors. Both the SNPs rs6356 and rs6357 were in weak LD with rs2070762, so it is reasonable that rs2070762 was associated with hypertension, whereas rs6356 and rs6357 were not. Haplotypes composed of adjacent SNPs are considered to have higher information content than single SNPs and thus have more power to explore the association between candidate genes and complex diseases [27–29]. These haplotype analyses have reinforced the results of the single locus analysis.

In addition to the genetic evidence, we have also provided functional evidence supporting a role for the C allele of rs2070762 in the relationship with essential hypertension. The luciferase reporter construct containing the C allele of rs2070762 produced significantly greater luciferase activity than the luciferase reporter construct containing the T allele (Figure 2B). Furthermore, our EMSA assay has clearly shown that unknown nuclear factor(s) bound specifically to the non-susceptible T allele, but not to the susceptible C allele. These results indicate the existence of unidentified transcriptional repressor(s), which form a DNA–protein complex with the T allele, but not the C allele, thus resulting in lower promoter activity. It seemed that the C allele of rs2070762 functioned as an enhancer in the absence of binding by unidentified transcriptional repressor(s).

Finally, how might the functional rs2070762 in intron 12 of the TH gene affect susceptibility to hypertension? Higher activity of the peripheral sympathetic nervous system, accompanied by higher TH activity is known to

play an important role in the pathogenesis of hypertension in humans as well as spontaneously hypertensive rats [30]. In the present study, significant enhanced transcriptional activity by the C allele of rs2070762 in the absence of binding by unidentified transcriptional repressor(s) may be involved in the subtle control of TH gene expression, and may have an effect on the activity of TH and the activity of the peripheral sympathetic nervous system. The enhanced activity of the peripheral sympathetic nervous system will result in increased levels of catecholamines, and eventually lead to the pathogenesis of hypertension. However, we cannot measure catecholamine levels, one of the important intermediate phenotypes of hypertension in patients with and without the associated SNP. Comparison of catecholamine levels in patients with the C or T allele of rs2070762 will contribute to interpreting the association between the functional variant of the TH gene and hypertension.

Intronic *cis*-elements, such as enhancers or repressors, have been reported for various genes [31–36]. However, only a few studies have reported polymorphisms in the intron region of genes that determine susceptibility to complex disease traits that might function as an enhancer or repressor regulating the expression level of the gene in pathology, including rs243480 in intron 3 of the Cullin1 (CUL1) gene significantly associated with rheumatoid arthritis [37], rs909253 in intron 1 of the LTA gene and rs7291467 in intron 1 of the LGALS2 gene associated with myocardial infarction [38,39]. Also, the intronic genetic marker in the TH gene functioned as a transcriptional regulatory element. TH01 microsatellite, the intronic polymorphic TCAT repeat in intron 1 of the TH gene, significantly associated with essential hypertension [9] and schizophrenia [6], is endowed with regulatory properties [40], and allelic variations of TH01 commonly found in humans have a quantitative silencing effect on the TH gene expression by binding to specific nuclear factors [41]. The present study and others [6,9,37–39] have established that polymorphisms within the intron region might contribute to susceptibility to a common disease.

Recently, Rao et al. [42] found that promoter variant C-824T (rs10770141) in the TH gene contributed to heritable alteration in multiple autonomic traits, biochemical and physiological, and the ultimate disease trait of hypertension. In the present study, TH promoter variants were not covered. Based on genotype data from HapMap (http://www.hapmap.org), no significant linkage disequilibrium between rs10770141 and rs2070762 existed ($r^2 = 0.11$). Thus the biological function of rs2070762 in intron 12 of the TH gene may be independent of rs10770141. These results implied the important role of the TH gene in the pathogenesis of hypertension.

In summary, the genetic and molecular data in the present study indicated association of a common, functional rs2070762 of intron 12 of the TH gene with essential hypertension in the Northern Chinese Han population. These findings suggest that the TH gene might be involved in the development of essential hypertension through functional genetic polymorphism. Nonetheless, additional research will be required to better define the functional significance of the intronic variant and to clarify the mechanism that explains epidemiologic associations involving the TH gene.

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