Association Study With 33 Single-Nucleotide Polymorphisms in 11 Candidate Genes for Hypertension in Chinese

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Abstract—Essential hypertension is considered to be a typical complex disease with multifactorial etiology, which leads to inconsistent findings in genetic studies. One possibility of failure to replicate some single-locus results is that the underlying genetics of hypertension are not only based on multiple genes with minor effects but also on gene–gene interactions. To test this hypothesis, a case–control study was constructed in Chinese subjects, detecting both single locus and multilocus effects. Eleven candidate genes were selected from biochemical pathways that have been implicated in the development and progression of hypertension, and 33 polymorphisms were evaluated in 503 hypertension patients and 490 age- and gender-matched controls. Single-locus associations, using traditional logistic regression analyses, and multilocus associations, using classification and regression trees and multivariate adaptive regression splines, were both explored in this study. Final models were selected using either Bonferroni correction or cross-validation. Three polymorphisms, *TH**rs2070762, *ADRB2**Q27E, and *GRK4**A486V, were found to be independently associated with essential hypertension in Chinese subjects. In addition to these individual predictors, a potential interaction of *CYP11B2-AGTR1* is also involved in the etiology of hypertension. These findings support the multigenic nature of the etiology of essential hypertension and propose a potential gene–gene interactive model for future studies. (***Hypertension*. 2006;47:1147-1154.**)

Key Words: hypertension, essential ■ case-control studies ■ genetics

Essential hypertension is considered to be a typical complex disease and is influenced by both genetic and environmental factors.[1] Some clinical end points, such as renal disease, coronary heart disease, and stroke, may be because of long-term or acute hypertension.[2] Because the biological process of hypertension involves multiple physiological pathways, each of which may be affected by multiple gene products, it is reasonable to expect that there are multiple gene variants predisposing individuals to susceptibility or resistance to hypertension.[3,4]

Although numerous genetic variations have shown association with hypertension, these associations are often not reproducible. For example, studies of the angiotensin-converting enzyme (ACE) insertion/deletion polymorphism, an extensively investigated variant, have a large number of both positive and negative reports.[5–11] These inconsistent findings might be explained in part by the genetic and environmental heterogeneity among different ethnic groups.[12,13] On the other hand, the failure to replicate some single-locus results might be because of an underlying genetic architecture in which gene–gene interactions are the norm rather than the exception.[14–16] That is, the effects of the variants under study might be masked by the effects of unstudied variant(s) that affect the phenotype, too.[17] Therefore, tests for joint effects of multiple candidate variants may provide more information in the search for hypertension susceptibility genes.

With regard to the biological process of blood pressure regulation, we focused on 11 candidate genes associated with: (1) renin–angiotensin–aldosterone system (RAAS), including *ACE*, angiotensin II receptor type I (*AGTR1*), and aldosterone synthase (*CYP11B2*)[15,18]; (2) sympathetic nervous system, including α-1 adrenergic receptor 1A (*ADRA1A*), β-2 adrenergic receptor (*ADRB2*), and tyrosine hydroxylase (*TH*)[4,19,20]; (3) lipoprotein metabolism, including lipoprotein lipase (*LPL*)[21]; (4) intracellular messengers, including G protein β polypeptide 3 (*GNB3*) and epithelium nitric oxide synthase (*NOS3*) [22,23]; and (5) sodium and electrolyte balance, including G protein–coupled receptor kinase 4 (*GRK4*) and protein kinase lysine–deficient 4 (*WNK4*)[24,25] (as shown in Table 1).

In the present study, a large-scale evaluation of 33 candidate gene polymorphisms was undertaken in a Han Chinese case–control cohort. We tested for single locus by using typical

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TABLE 1. Genetic Polymorphisms Evaluated in This Study

Pathway	Gene	Symbol	Chromosome	Polymorphisms	Allele*	MAF†	Description and Potential Function‡
RAAS	Aldosterone synthase gene	*CYP11B2*	8q21	T-344C	T/C	0.32	Putative binding site for SF-1[1]
				L173R	A/G	0.33	Lys to Arg at codon 173[2]
				Intron2 Conversion (IC)	W/C	0.17	An intronic conversion in intron 2[1]
	Angiotensin II type 1 receptor	*AGTR1*	3q21-25	A-1138T	A/T	0.16	Perfect LD with T-810A§ [3]
				C-521T	C/T	0.21	Associated with platelet AII binding[4]
				A1166C	A/C	0.06	3′ UTR[5]
	ACE	*ACE*	17q23	Ins/Del	I/D	0.36	Ins/Del in intron 16, associated with plasma ACE levels[6]
Sympathetic nervous system	α-1 adrenergic receptor 1A	*ADRA1A*	8p22	C2564T	T/C	0.14	3′ UTR
				G2547C	C/G	0.01	3′ UTR
				C2254G	G/C	0.46	3′ UTR
				C2238T	T/C	0.08	3′ UTR
				T1991C	C/T	0.04	3′ UTR
				E465D	C/T	0.04	Glu to Asp at codon 465
				R347C	A/T	0.15	Arg to Cys at codon 347[7]
	β-2 adrenergic receptor	*ADRB2*	5q31-32	T-47C	T/C	0.12	Modulating mRNA translation[8]
				Q27E	C/G	0.10	Gln to Glu at codon 27[9]
				R16G	A/G	0.39	Arg to Gly at codon 16[9]
	Tyrosine hydroxylase	*TH*	11p15.5	rs2070762	T/C	0.42	Potential LD with other functional locus[10]
				rs6356	A/G	0.21	Val to Met at codon 81[10]
				rs6357	G/A	0.07	Lys to Lys at codon 240[10]
Lipoprotein metabolism	Lipoprotein lipase	*LPL*	8p22	S447X	C/G	0.08	Ser to stop at codon 447[11]
				P5	C/T	0.35	Intron 4
				P8	C/A	0.12	Thr to Thr at codon 388[11]
Intracellular messengers	Epithelium NO synthase	*NOS3*	7q36	T-786C	T/C	0.09	Associated with reduced promoter activity[12]
				INTRON4	5/4	0.09	A VNTR polymorphism in introns 4¶[13]
				G894T	G/T	0.11	Glu to Asp at codon 298[13]
	G protein β polypepide 3	*GNB3*	12p13	C825T	C/T	0.47	Associated with a splicing variant[14]
				C1429T	C/T	0.19	3′ UTR, strong LD with C825T[14]
				A-350G	G/A	0.02	Associated with a binding motif for E-box[14]
Sodium-electrolyte balance	G protein–coupled receptor kinase 4	*GRK4*	4p16.3	R65L	G/T	0.13	Arg to Leu at codon 65[15]
				A486V	T/C	0.43	Ala to Val at codon 486[15]
				A142V	C/T	0.21	Ala to Val at codon 142[15]
	Protein kinase, lysine-deficient 4	*WNK4*	17q21-22	G1662A	G/A	0.03	Ala to Ala at codon 547[16]

SF-1 indicates steroidogenic factor-1; UTR, untranslated region; VNTR, variable number of tandem repeats.
*Major allele/minor allele.
†Minor allele frequency in controls.
‡See online references.
§The SNP T-810A might destroy a transcription factor binding site for GATA binding factors.
¶VNTR: 4 or 5 repeats of 27 bp.

logistic regression, as well as multilocus association using 2 statistical methods: classification and regression trees (CART)[26] and multivariate adaptive regression splines (MARS).[27] Both single locus and multilocus analyses were applied to test our hypothesis that these candidate genes under study may contribute to the etiology of hypertension independently and/or through complex interactions.

Methods

Study Population

All of the DNA samples and clinical data for participants in this study were collected from the International Collaborative Study of Cardiovascular Disease in Asia.[28] The protocol was approved by the local bioethical committee, and informed consent was obtained from each participant. From the nationally representative sample of the

International Collaborative Study of Cardiovascular Disease in Asia,[28] we selected 503 unrelated stage-2 hypertensive patients with an average systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥100 mm Hg and 490 age- and gender-matched unrelated healthy control subjects. Three blood pressure measurements were obtained according to a standard protocol recommended by the American Heart Association.[29] Subjects with a clinical history of secondary hypertension, coronary heart disease, and diabetes were excluded from this study.

Selection of Candidate Genes and Polymorphisms

We selected 11 candidate genes from biochemical pathways that have been implicated in the development and progression of hypertension. We selected 33 single-nucleotide polymorphisms (SNPs) of these genes based on previous evidence of potential functionality, validated allele frequency, and sequence-proven allelic variation. Detailed information of 11 candidate genes and 33 SNPs is shown in Table 1.

DNA was extracted from leukocytes using a standard phenol–chloroform method. All of the SNPs were genotyped using standard polymerase chain reaction/restriction fragment length polymorphism or direct sequencing methods.

Statistical Analyses

We sought evidence of association between each of the 33 SNPs, as well as their interactions and hypertension. Genomic control was used to examine the potential impact of population structure (λ) in this study.[30,31] To avoid the possible correlations among SNPs in each candidate gene, only 1 SNP was randomly selected from those genes with multiple markers. This process was repeated 1000 times, and the mean value of λ was calculated. Our data showed that the mean effect was 1.12, indicating that no correction was necessary.

Single Locus Analyses

First, for descriptive purposes, crude allele and genotype frequencies for each SNP were calculated, and Hardy–Weinberg equilibrium was evaluated by using a goodness-of-fit test in controls. To avoid assumptions regarding modes of inheritance, all of the analyses were performed using additive, dominant, or recessive modes of each SNP. SNPs with a nominal $P<0.1$ were presented in the initial results. Second, we performed forward-stepwise multivariable logistic regression analyses using a nominal P value cut point of 0.01 to evaluate the independent effect of each SNP on hypertension. Multiple testing was adjusted using the Bonferroni correction.

The pattern of pairwise linkage disequilibrium (LD) between SNPs within each candidate gene was measured by D' and r^2, using the software GOLD.[32] The haplo.score approach, as outlined by Schaid et al,[33] was performed to assess the potential effects of haplotypes within each gene. This method models an individual's phenotype as a function of each inferred haplotype, weighted by their estimated probability, to test the global effects of haplotypes, as well as the individual effect.

Multilocus Analyses

The programs of CART and MARS (Salford Systems) were used to test for potential gene–gene interaction and thereby to identify specific locus combinations of interest for further investigation and replication.

CART[26] can evaluate the relative significance of each predictor, and iteratively subdivides data to build a hierarchical classification model for an optimal combination of independent variables. The strength of CART is its ability to detect high-level interactions among the predictor variables. We ran the program with the following parameters: Gini index as a splitting criterion; a maximum tree depth of 4, indicating that 4-way interaction was allowed; and a minimum terminal node size of 50, ensuring that the null expected number of cases per terminal node would be ≥25.

MARS[27] is a generalization of stepwise linear regression that is particularly suited for high-dimensional problems in which many independent variables might be modeled. MARS has the advantage that additive as well as interactive effects can be included in the models. In this study, 1-way (individual effect), 2-way, 3-way, and 4-way interaction models were considered. The maximum number of basis functions for each model was set to 20, 30, 40, and 60, respectively.

In both CART and MARS, the optimal models were selected in a similar 2-stage process. First, an overly large model was to fit the data by adding new nodes (CART) or basis functions (MARS). Second, the overfitted model was pruned back to a more optimal size. We used 10-fold cross-validation to evaluate overall model fit. The CART or MARS model was developed using randomly divided nine tenths of the data and then evaluated on the remaining one tenth of the subjects. To reduce the variability because of the random stratification into 10 strata, the 10-fold cross-validation process was repeated, and the results were averaged.

Results

Table 1 lists the 11 candidate genes and 33 SNPs examined and the observed frequency of minor allele at each site among the control subjects, as well as the description of potential functions. All of the sites were in Hardy–Weinberg equilibrium in controls after Bonferroni correction except for SNP *TH**rs2070762. We retyped this polymorphism in 98 randomly selected individuals and found 100% concordance with our original genotyping score, indicating that the genotyping error could be excluded.

The demographic and clinical characteristics of all of the individuals are shown in Table 2. The case group had significantly higher body mass index, systolic blood pressure, diastolic blood pressure, serum total cholesterol levels, triglyceride levels, and glucose levels than the controls, as well as lower high-density lipoprotein cholesterol levels. There were no significant differences between the cases and controls for smoking and drinking status.

TABLE 2. Comparison of Clinical Characteristics Between Cases and Controls

Variables	Case (n=503)	Control (n=490)	*P*
Gender, M/F	262/241	257/233	0.91
Age, y	53.6±9.3	53.5±9.2	0.92
BMI, kg/m^2	26.3±3.85	24.30±3.56	<0.0001
SBP, mm Hg	177.1±28.0	117.5±11.6	<0.0001
DBP, mm Hg	104.3±12.3	75.0±8.0	<0.0001
TC, mmol/L	5.22±0.98	5.06±1.05	0.01
TG, mmol/L	1.70±1.06	1.43±0.86	<0.0001
CR, μmol/L	70.8±15.2	68.9±12.1	0.03
HDL_C, mmol/L	1.25±0.30	1.32±0.34	0.001
LDL_C, mmol/L	3.19±0.85	3.09±0.87	0.05
GLU, mmol/L	5.93±1.79	5.59±1.68	0.001
Smoker, %*	204 (40.6)	211 (43.1)	0.42
Drinker, %†	173 (34.4)	164 (33.5)	0.76

Values are mean±SD unless otherwise specified. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; CR, creatinine; HDL_C, high-density lipoprotein cholesterol; LDL_C, low-density lipoprotein cholesterol; Glu, glucose.

*Smokers, the No. of cigarette consumers who has smoked ≥100 cigarettes.

†Drinkers, the No. of alcohol consumers who drank ≥12 times during the year ahead of the interview.

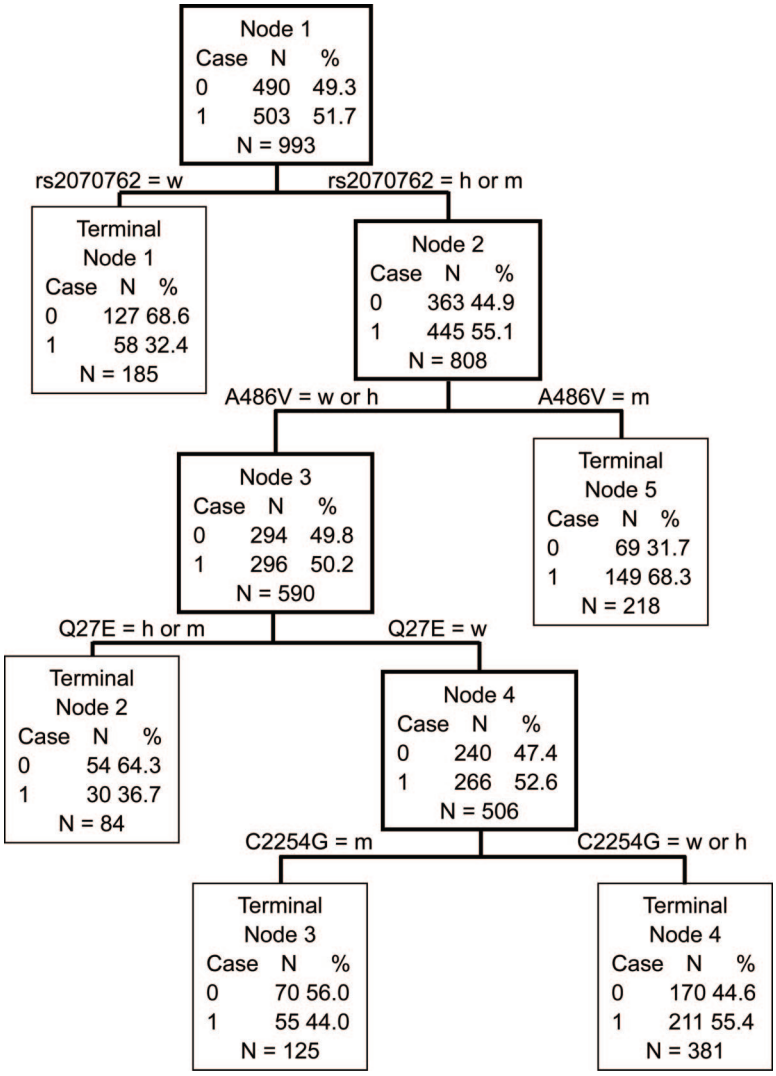
TABLE 3. Estimated Effects for Polymorphisms Selected in Univariable and Forward-Stepwise Multivariable Analyses for Hypertension

			Crude			Stepwise Multivariable			
Gene	Polymorphism	Model	OR	95% CI	*P*	OR	95% CI	*P*	Bonferroni
CYP11B2	L173R	Additive	0.78	0.64 to 0.95	0.014				
		Recessive	0.55	0.35 to 0.87	0.01				
	IC	Additive	1.39	1.11 to 1.74	0.004				
		Dominance	1.40	1.07 to 1.82	0.013				
		Recessive	2.16	1.11 to 4.23	0.024				
ADRA1A	C2564T	Additive	0.77	0.59 to 1.01	0.056				
	G2547C	Additive	2.74	1.23 to 6.12	0.014				
		Dominance	2.76	1.21 to 6.25	0.015	3.63	1.43 to 9.23	0.007	0.198
	C347R	Additive	0.76	0.58 to 0.99	0.042				
		Dominance	0.78	0.58 to 1.04	0.089				
ADRB2	Q27E	Additive	0.56	0.40 to 0.78	0.001				
		Dominance	0.45	0.31 to 0.66	<0.0001	0.45	0.30 to 0.68	0.0001	0.004
	R16G	Additive	1.24	1.05 to 1.48	0.014				
		Dominance	1.38	1.06 to 1.79	0.015	1.46	1.11 to 1.93	0.008	0.228
TH	rs2070762	Additive	1.60	1.25 to 2.05	0.0002				
		Dominance	2.68	1.91 to 3.77	<0.0001	2.86	1.99 to 4.10	1.17×10^{-8}	3.9×10^{-7}
GNB3	A-350G	Additive	2.23	1.24 to 4.01	0.007				
		Dominance	2.22	1.21 to 4.06	0.01				
GRK4	A486V	Additive	1.59	1.34 to 1.89	<0.0001				
		Dominance	1.74	1.32 to 2.30	<0.0001				
		Recessive	2.08	1.55 to 2.77	<0.0001	2.05	1.50 to 2.79	5.10×10^{-6}	0.0002
	A142V	Additive	0.80	0.64 to 1.00	0.046				
		Dominance	0.79	0.61 to 1.03	0.08				

Pairwise LD coefficients were displayed in Table I, available in an online supplement at http://hyper.ahajournals.org. Genotype frequencies for cases and controls, as well as odds ratios (OR) associated with additive, dominant, or recessive modes of inheritance for each of the 33 individual polymorphisms, were calculated. Table 3 presents the finding with a nominal univariable $P<0.1$ for association with hypertension. As shown, 11 polymorphisms were found in this initial analysis. In the following forward-stepwise multivariable logistic regression analyses, the *ADRA1A**G2547C polymorphism (OR, 3.63; 95% CI, 1.43 to 9.23; $P=0.0067$), *ADRB2**Q27E polymorphism (OR, 0.45; 95% CI, 0.298 to 0.68; $P=0.0001$), *ADRB2**R16G polymorphism (OR, 1.46; 95% CI, 1.11 to 1.93; $P=0.0078$), *GRK4**A486V polymorphism (OR, 2.05; 95% CI, 1.50 to 2.79; $P=5.1\times10^{-6}$), and *TH** rs2070762 polymorphism (OR, 2.86; 95% CI, 1.99 to 4.10; $P=1.17\times10^{-8}$) were found to be independent predictors of hypertension. After the conservative Bonferroni correction (simultaneous adjustment for 33 comparisons), the dominant effects of *ADRB2**Q27E and *TH**rs2070762 and the recessive effect of *GRK4**A486V remained significant associations with hypertension, indicating that these 3 SNPs are likely to represent independent effects.

Consistent with the single locus results, the haplotype analysis also found that only the *ADRB2* gene, *TH* gene, and *GRK4* gene showed significant effects for global haplotypes after Bonferroni correction (adjustment for 11 candidate genes). For instance, the haplotypes with the A486V C allele within the *GRK4* gene had higher frequencies in cases than in controls, whereas the haplotypes with the A486V T allele had lower frequencies. Similar results were observed in the *ADRB2* and *TH* genes (See Table II, available online).

The final CART model selected after pruning is shown in the Figure. The number and percentage of cases (case=1) and controls (case=0) are shown for each node. Five terminal nodes were fit. As shown in the Figure, the 3 most important predictors were the polymorphisms selected in the logistic model. The first split was according to the *TH**rs2070762 genotype, indicating a dominant effect. Those with the heterozygous and homozygous mutant genotypes (C allele carriers) were further split according to the *GRK4**A486V genotype, in a recessive effect. The next 2 split nodes were *ADRB2**Q27E and *ADRA1A**C2254G, in dominant and recessive effects, respectively. With those in terminal node 1 serving as the reference group, Table 4 presents naive ORs for terminal nodes 2, 3, 4, and 5. These results suggested that *TH**rs2070762 exerted the greatest impact on hypertension risk, followed by *GRK4**A486V and *AGRB2**Q27E. The CART model also indicated some potential interactions among *TH*, *GRK4*, *ADRB2*, and *ADRA1A* genes. For example, the combination of *TH**rs2070762 C allele and *GRK4**A486V VV genotype significantly increased the risk of hypertension (Figure, terminal



CART model for hypertension. (Genotypes are denoted as w, homozygous wild genotype; h, heterozygous genotype; and m, homozygous mutant.

node 5). However, some decreased risk of hypertension was found among the subjects with *TH**rs2070762 C allele, *GRK4**A486V A allele, and *AGRB2**Q27E E allele (Figure, terminal node 2).

The final 1-way model chosen using the MARS was completely consistent with the logistic model. The individual dominant effects of *TH**rs2070762 and *ADRB2**Q27E and recessive effect of *GRK4**A486V were found to be independently associated with hypertension (Table 4). The MARS model with 2-way interactions included 2 additive interactions of *TH**rs2070762(hm) and *ADRB2**Q27E(w) and *GRK4**A486V(m) and *GNB3**A-350G(hm). Here, *w* denotes the homozygous wild genotype, *h* denotes heterozygous genotype, and *m* denotes homozygous mutant genotype. The naive ORs are shown in Table 4. When 3-way interactions were allowed, a different MARS model was selected. This model contained a dominant effect of *ADRB2**Q27E(w); three 2-way interactions: *GRK4**A486V(m)-*GNB3**A-350G(hm), *TH**rs2070762(hm)-*GNB3**A-350G(hm), and *CYP11B2**IC(hm)-*GRK4**A486V(m); and a 3-way interaction of *ADRB2**R16G(hm)-*TH**rs2070762(hm)-*GNB3**A-350G(hm) (Table 4). Although 4-way interactions were allowed, none were selected in the final model (data not shown).

We also used logistic regression to fit the models including all of the terms selected in the MARS models, as well as all of the lower-order interactions and individual effect terms. For 2-way interaction models, however, when the individual effect terms (*TH**rs2070762, *ADRB2**Q27E, *GRK4**A486V, and *GNB3**A-350G) were included in the model, the interactions of these terms were no more significant, indicating that these interactions depend on the individual effect terms. The similar results were observed in the 3-way interaction model, except for an interaction of *CYP11B2* and *GRK4*, with marginal significance ($P=0.09$; data not shown).

To investigate whether there are interactions among those polymorphisms displaying no individual effects on hypertension, we further constructed the models excluding those 3 SNPs with individual effects (*TH**rs2070762, *ADRB2**Q27E, and *GRK4**A486V). Only one 2-way interaction, *CYP11B2**IC(hm)-*AGTR1**A-1138T(w), was found to be significantly associated with hypertension. This interaction effect was supported by the crude data (Table 5). When the subjects were stratified by the *CYP11B2**IC polymorphism, there was an effect of the *AGTR1**A-1138T polymorphism only among those with the IC C allele. Compared with the

TABLE 4. Final Models and Naive Estimates of OR, CI, and *P* Values Using CART and MARS

Predictor	OR	95% CI	*P*
CART model			
Terminal node 2 vs terminal node 1	1.22	0.68 to 2.16	0.48
Terminal node 3 vs terminal node 1	1.72	1.05 to 2.83	0.02
Terminal node 4 vs terminal node 1	2.72	1.85 to 4.01	<0.0001
Terminal node 5 vs terminal node 1	4.73	3.04 to 7.38	<0.0001
MARS model: 1-way			
*TH**rs2070762(hm)	2.69	1.90 to 3.81	<0.0001
*GRK4**A486V(m)	2.05	1.52 to 2.75	<0.0001
*ADRB2**Q27E(hm)	0.48	0.33 to 0.71	<0.0001
MARS model: 2-way			
*TH**rs2070762(hm)-*ADRB2**Q27E(w)	2.73	2.04 to 3.65	<0.0001
*GRK4**A486V(m)-*GNB3**A-350G(hm)	2.25	1.69 to 3.00	<0.0001
MARS model: 3-way			
*ADRB2**Q27E(hm)	0.46	0.31 to 0.68	<0.0001
*TH**rs2070762(hm)-*GNB3**A-350G(hm)	1.96	1.29 to 2.99	0.002
*GRK4**A486V(m)-*GNB3**A-350G(hm)	1.70	1.22 to 2.38	0.002
*CYP11B2**IC(hm)-*GRK4**A486V(m)	1.55	1.15 to 2.10	0.004
*ADRB2**R16G(hm)-*TH**rs2070762(hm)-*GNB3**A-350G(hm)	3.10	1.43 to 6.70	0.004
Excluded *TH**rs2070762, *GRK4**A486V, and *ADRB2**Q27E			
MARS model: 2-way			
*CYP11B2**IC(hm)-*AGTR1**A-1138T(w)	1.74	1.30 to 2.34	0.0002

Genotypes are denoted as w, homozygous wild genotype; h, heterozygous genotype; and m, homozygous mutant.

*AGTR1**A-1138T T allele carriers, the OR was 2.10 (95% CI, 1.26 to 3.51; $P=0.002$) for those individuals with AA homozygotes. The logistic regression analysis also indicated that this interaction was independent of the other 3 individual predictors (Table 6).

Discussion

In the present study, we examined the relation of 33 polymorphisms in 11 candidate genes with the risk of hypertension. Both single-locus and multilocus analyses revealed that 2 genes from the sympathetic system (*TH* and *ADRB2*) and 1 gene affecting the sodium balance (*GRK4*) were independently associated with the significant risk of hypertension in the Chinese Han population. In addition to these 3 individual predictors, an interaction between *CYP11B2* and *AGTR1*, both from RAAS, was also found to be involved in the relationship with hypertension. These findings support the recognized understanding that complex genetic interactions account for hypertension risk and propose a potential gene–gene interactive model.

To control for false-positive findings, several approaches were considered. First, we included only Northern Han Chinese subjects, who have been proven to have a significantly higher incidence and prevalence of hypertension and higher mean levels of blood pressure.[28,34] Our genomic control analysis revealed no evidence of population stratification in this data. Second, all of the candidate genes selected have a substantial priori probability of involvement in hypertension. Finally, we used conservative Bonferroni correction and 10-fold cross-validation in single-locus and multilocus analyses, respectively, to control the false-positive findings potentially because of the multiple testing and overfitted model.

Although further functional studies are necessary to fully understand the underlying biological mechanisms of the observed genetic associations, our findings are biologically plausible. There are numerous studies demonstrating that

TABLE 5. Joint Genotype Frequencies for 2 Polymorphisms Selected by MARS After Excluding Individual Effect Predictors

	*CYP11B2**IC-WW			*CYP11B2**IC-WC or CC		
Polymorphism	Cases, n (%)	Controls, n (%)	*P*	Cases, n (%)	Controls, n (%)	*P*
*AGTR1**A-1138T						
AA	221 (71.75)	243 (72.54)	0.82	148 (78.72)	95 (63.76)	0.002
AT or TT	87 (28.25)	92 (27.46)		40 (21.28)	54 (36.24)	

TABLE 6. Logistic Regression Model Using 3 Individual Predictors and Interaction of *CYP11B2-AGTR1*

Polymorphisms	Coefficient	SE	OR	95% CI	*P*
*ADRB2**Q27E	−0.821	0.205	0.44	0.29 to 0.66	0.0001
*TH**rs2070762	1.024	0.181	2.79	1.95 to 3.97	1.57×10^{-8}
*GRK4**A486V	0.745	0.154	2.11	1.56 to 2.85	1.26×10^{-6}
*CYP11B2**IC-*AGTR1**A-1138T	0.572	0.157	1.77	1.30 to 2.41	0.0003

essential hypertension is accompanied by sympathetic activation.[4,19,20] It has been shown that noradrenaline plays an important role in the regulation of blood pressure, and the increased plasma catecholamine levels have been observed in the development of hypertension. As the rate-limiting enzyme in catecholamine biosynthesis, the *TH* gene is considered as a logic candidate for the etiology of hypertension.[4,35] The present study found a SNP (rs2070762) located in intron 13 strongly associated with an increased risk of hypertension.

As an important component of sympathetic system, the *ADRB2* gene has been implicated in the pathogenesis of hypertension, on the basis of both studies, suggesting altered β2-mediated changes of cardiovascular functions[36] and molecular genetic studies.[37] Results from linkage and association studies support this gene participating in blood pressure regulation and development of hypertension.[37–39]

With regard to *GRK4*, this gene has been implicated in essential hypertension, because it participates in the desensitization of the D_1 receptor, which leads to sodium retention.[24] The role of the *GRK4* gene in the dopaminergic system in hypertension has recently been thoroughly reviewed.[40,41] The current data indicated that *GRK4**A486V was associated with hypertension in recessive mode, after Bonferroni correction, which was consistent with previous reports in white subjects.[42]

We previously reported linkage and association with hypertension and blood pressure on chromosome 8p22 in our hypertensive families.[21] Two candidate genes for hypertension in this region, *ADRA1A* and *LPL*, were included in this study. No association with hypertension was observed for *LPL* gene polymorphisms. However, we found that a novel SNP, C2547G, located in the 3′-untranslated region of *ADRA1A* gene, was significantly associated with hypertension before Bonferroni correction, with the minor G allele more frequently observed in cases (2.3%) than in controls (0.8%). Because of the low frequency of the minor allele, this association should be interpreted with caution.

Our further exploratory multilocus analyses provided some suggestive joint effects of *TH-ADRB2*, *TH-GNB3*, *GRK4-GNB3*, and *TH-ADRB2-GNB3*. An interesting finding is the interaction of *CYP11B2* and *GRK4*, with marginal significance ($P=0.09$) in logistic regression. A recent study constructed in Ghanaian subjects, including 13 polymorphisms of 8 genes, reported an interaction of *GRK4* and *ACE* associated with hypertension.[43] Among Japanese subjects, the best combination that was predictive of hypertension included *GRK4*, *ACE*, and *CYP11B2*; however, for low-renin hypertension in Japanese subjects, the best genetic model was also reported, which included only *GRK4* and *CYP11B2*.[41] Williams et al[43] indicated that robustness for blood pressure might be maintained by genes in different but potentially compensatory pathways, vasoconstriction and sodium balance.

To investigate the potential interactions among those polymorphisms displaying no individual effects on hypertension, we further constructed the models excluding those 3 individual predictors (*TH*rs2070762, *ADRB2**Q27E, and *GRK4**A486V). An interactive effect on hypertension was found between *CYP11B2**IC and *AGTR1**A-1138T, both from RAAS. This joint effect was supported by the crude data (Table 5) and logistic regression analysis (Table 6). The effects of combinations of RAAS gene polymorphisms on blood pressure and hypertension have also been investigated in previous studies, in which the combinations of polymorphisms were associated with the risk of hypertension, although no individual effect of each isolated genotype was detected.[15,16] Recent studies indicated that the *CYP11B2* gene and protein, locally presented in kidney, were regulated by low salt intake and angiotensin II type 1 receptor.[44] These findings suggested an epistatic interaction in these 2 or more candidate genes in RAAS.

This study attempted to apply advanced statistical methods to address 2 common problems encountered in genetic dissection of complex traits. The first is the multiple testing, which is almost inevitable in large-scale gene mapping efforts. One approach used in single-locus analyses was conservative Bonferroni correction to control for the false-positive results. The other approach used in multilocus analyses was cross-validation to prune the overfitted model. The second is the high-dimensional problem caused by gene-gene interaction, which has been widely accepted as an important contributor to the complexity of mapping complex disease genes.[17] In the present study, we used CART and MARS to fit the potential interactive models. Both methods offer advantages over traditional logistic regression in that they may discover interactions of genes displaying no strong individual effects[45] and have been used in genetic association studies.[46–48]

Perspectives

The present study provides further evidence that several functional polymorphisms within candidate genes act individually or together in the etiology of essential hypertension. Our data also demonstrate the use of nonparametric methods, such as CART and MARS, in detecting a gene–gene interaction effect for a complex disease. Further replications in larger independent samples are warranted. In addition, functional studies to prove the true existence of an interaction between the *CYP11B2* and *AGTR1* genes are also required in the future.

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