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Association of −344C/T polymorphism in the aldosterone synthase (CYP11B2) gene with cardiac and cerebrovascular events in Chinese patients with hypertension

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Abstract

Objective: Several recent studies have shown that the aldosterone synthase gene (*CYP11B2*) −344C/T polymorphism is related to cardiovascular diseases. However, whether the −344C allele influences the incidence of cardiovascular diseases in Chinese patients with hypertension is unclear.

Methods: Chinese patients with essential hypertension were genotyped for the −344C/T polymorphism in *CYP11B2* (n = 755; CC, n = 112; CT, n = 361; TT, n = 282) and followed for 11 years for major adverse cardiovascular events (MACEs), including stroke, onset of coronary artery disease (CAD), and CAD-related death. Established cardiovascular risk factors were used to adjust the multivariate Cox analysis.

Results: After a mean follow-up period of 7.60 ± 1.12 years, a significantly higher incidence of MACEs was seen in patients with the CC genotype than in those with the CT and TT genotypes. The CC variant was significantly and independently predictive of MACEs (hazard ratio = 2.049), CAD (hazard ratio = 1.754), and stroke (hazard ratio = 2.588), but not CAD-related stroke or death.

Conclusion: The *CYP11B2* −344 CC genotype is a risk factor for CAD and stroke, independent of other established cardiovascular risk factors in Chinese patients with hypertension.

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Introduction

Essential hypertension (EH) is a complicated, chronic, noncommunicable disease that is a major risk factor for cardiovascular disease and mortality, including ischemic heart disease[1] and stroke.[2] Annually, approximately 9.4 million deaths are attributed to high blood pressure, which is one of three leading risk factors for global disease burden.[3,4] Predicting the development of cardiovascular diseases in patients with hypertension is important.

The human aldosterone synthase (*CYP11B2*) gene is located at chromosome 8q22.[5] It encodes a key enzyme involved in the terminal steps of aldosterone biosynthesis.[6] One study from Egypt suggests that *CYP11B2* genotypes and baseline serum K are predictors of spironolactone response in heart failure with reduced ejection fraction, which suggests that it may be correlated with cardiovascular disease.[7] In the 30 years since it was first sequenced, numerous common *CYP11B2* polymorphisms have been identified. The most studied is the rs1799998 C/T single nucleotide polymorphism (SNP), also referred to as "−344" because of its base position relative to the gene transcription start site.[8,9] At the molecular level, the role of the *CYP11B2* locus has been extensively evaluated in cardiovascular disease, with particular attention to the −344C/T SNP. Previous studies also identified variants of *CYP11B2* associated with increased risk of EH.[10,11] We found that the −344C allele is strongly associated with increased left ventricular size and decreased baroreflex sensitivity in healthy individuals; these physiological parameters are cardiovascular risk factors.[8,12] Nevertheless, one report suggested that rs1799998 is not associated with hypertension or other cardiovascular indicators in a population in Thailand.[13]

Recent population-based studies established the association of the −344C/T *CYP11B2* polymorphism with the risk of cardiovascular diseases.[14,15] Because of differences in −344C/T allele frequencies and incidences of cardiovascular disorders in different ethnicities, these results may not be applicable to the Chinese population. Moreover, most of these studies were conducted in healthy populations and, at least in part, were unable to demonstrate a genetic association for patients with hypertension. Therefore, whether the allele at −344C/T influences the incidence of cardiovascular disease in patients with hypertension needs to be confirmed in hypertensive cohort-based studies. Here, we conducted a prospective study to investigate the association between the −344C/T SNP and the risk of cardiovascular diseases in a cohort of Chinese individuals with hypertension.

Methods

Ethical approval

The protocol was approved by the Institutional Ethics Committee of Hebei General Hospital and Beijing Xuanwu Hospital, China. Written informed consent was obtained from each study participant.

Study subjects

In this study, 820 outpatients diagnosed as having EH without cardiovascular disease were recruited sequentially between June 2004 and May 2006 at Hebei General Hospital and Beijing Xuanwu hospital. A clinical survey and genotyping were conducted for each patient. Sixty-five patients were excluded because of lack of follow-up. The remaining 755 patients were included (CC, n = 112; CT, n = 361; TT, n = 282; Table 1). Diagnosis of EH was based on the 8th report of the Joint National Committee. Exclusion criteria were present or past ischemia (patients underwent exercise electrocardiography, stress myocardial perfusion imaging, or coronary artery angiography, and showed no evidence of myocardial ischemia), congenital or valvular heart disease, dilated or hypertrophic cardiomyopathy, myocarditis, heart failure, stroke, and other cardiovascular or cerebrovascular diseases.

Measurements of cardiovascular disease risk factors

At study entry, blood pressure was taken at two office visits at least 1 week apart by the same trained nurse using a standard mercury sphygmomanometer with the patient in the sitting position. The measurement was taken using the right arm with an appropriate cuff size and after the subject had rested for at least 5 minutes. Systolic and diastolic blood pressures were based on the first and fifth Korotkoff phases, respectively. Three measurements were made at 2-minute intervals and the mean was used in the analysis.

Table 1. Study participant characteristics.

	CYP11B2 genotype			
	CC	CT	TT	*p*-value
Total number, n (%)	112 (14.8)	361 (47.8)	282 (37.4)	
Sex [male, n (%)]	51 (45.54)	196 (54.29)	152 (53.90)	0.243
Age (years)	47.74 ± 16.58	44.67 ± 15.85	43.23 ± 15.19	0.299
Blood pressure				
Systolic blood pressure (mmHg)	151.38 ± 12.50	149.44 ± 14.69	147.11 ± 13.09	0.095
Diastolic blood pressure (mmHg)	97.51 ± 7.37	97.95 ± 9.68	97.12 ± 8.24	0.718
Current or former smoker, n (%)	38 (33.93)	147 (40.72)	95 (33.67)	0.141
Body mass index, kg/m^2	26.24 ± 3.21	26.08 ± 3.57	26.22 ± 3.16	0.847
Diabetes, n (%)	18 (16.07)	59 (16.34)	31 (10.99)	0.133
Hyperlipidemia, n (%)	69 (61.61)	226 (62.60)	179 (63.48)	0.938
Total cholesterol, mg/dL	197.18 ± 59.81	195.06 ± 63.52	195.03 ± 59.32	0.945
HDL cholesterol, mg/dL	62.07 ± 20.37	62.64 ± 19.36	63.75 ± 19.96	0.700
LDL cholesterol, mg/dL	94.85 ± 45.07	98.13 ± 47.68	92.23 ± 46.80	0.324
Triglycerides, mg/dL	178.95 ± 136.11	175.77 ± 132.02	185.92 ± 175.09	0.713
Uric acid, mg/dL	5.11 ± 1.65	5.31 ± 1.66	5.12 ± 1.59	0.370
Family history of hypertension, n (%)	60 (53.6)	213 (59)	146 (51.8)	0.170
Antihypertensive agents				
Calcium channel blocker, n (%)	6 (5.4)	18 (5)	12 (4.3)	0.895
ACEI/ARB, n (%)	26 (23.2)	75 (20.8)	70 (24.8)	0.471
Beta blockers, n (%)	4 (3.6)	6 (1.7)	5 (1.8)	0.399
Diuretics, n (%)	11 (9.8)	20 (5.5)	18 (6.4)	0.274

HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker.

Body mass index (BMI, kg/m^2) was calculated from standardized measurements of height and weight. Current smoking was defined as having smoked at least 100 cigarettes in the patient's lifetime and currently smoking on all or some days during the past year. Diabetes was defined as fasting plasma glucose $\geq$7.0 mmol/L (126 mg/dL) or glycated hemoglobin (HbA1c) $\geq$6.5% according to the American Diabetes Association criteria or reported use of diabetic medications.

All blood biochemical markers were derived from the existing medical record or, where this was unavailable for the preceding 12 months, by measurement during the screening phase of the study. Disease status, personal history of cardiovascular diseases, and antihypertensive or other medication use were reported by the usual treating physician.

Genotyping

Venous blood was collected in 5-mL EDTA tubes. Genomic DNA was isolated with the QIAamp DNA Blood Mini Kit (Qiagen, Hamburg, Germany) and stored at −20°C. The *CYP11B2* −344C/T polymorphism was determined by PCR-restriction fragment length polymorphism (RFLP). The primers used were 5′-CAGGGCTGA GAGTAAAA-3′ (forward) and 5′-CAG GGGGTACGTGGACATTT-3′ (reverse) as previously reported.[16] The PCR amplification was performed in a 20-μL reaction mixture (PCR master mix, TaKaRa Bio, Tokyo, Japan) with 2 μL of template DNA. The thermal cycling conditions included initial denaturation for 5 minutes at 95°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s, extension at 72°C for 30 s, and final extension for 10 minutes at 72°C. The amplicon was digested with HaeIII (2.5 units) overnight and subjected to 3% agarose gel electrophoresis. The digestion of the 153-bp PCR product resulted in the production of 97- and 56-bp bands in the presence of the C allele, whereas the product remained uncut in presence of the T allele. In addition to RFLP analysis, the selected PCR products were clone into pGEM-T Easy Vector Systems (Promega, Madison, WI, USA), and gene sequencing was performed to confirm the polymorphisms.

Clinical follow-up

Clinical follow-up was conducted by face-to-face interview in the consulting room, telephone contacts, or clinical visits (electronic medical record) every September. The questionnaire included events related to hypertensive complications and occurrence of death. We confirmed the responses by comparing them against patients' medical records. The primary endpoint of this study was occurrence of major adverse cardiovascular events (MACEs), which included stroke (defined as rapidly developing signs of focal or global disturbance of cerebral function lasting >24 hours or leading to death, with no apparent cause other than that of vascular origin and diagnosis with computed tomography and/or magnetic resource imaging), the onset of coronary artery disease (CAD, including angina pectoris, myocardial infarction, and cardiovascular death). The follow-up duration was considered to encompass the interval from initial evaluation to the time of event onset or June 2018. The average follow-up period was 7.60 ± 1.12 years.

Statistical analyses

Categorical variables are reported as counts and percentages; continuous variables are presented as means ± standard deviations. Differences in proportions were statistically evaluated using the Chi-square test. Continuous variables were compared using ANOVA for parametrically distributed

variables or Kruskal–Wallis statistics for nonparametrically distributed variables. The genotype and allelic frequencies of the C344T *CYP11B2* were assessed by Hardy-Weinberg equilibrium (HWE) and compared by Chi-square test and Fisher's exact test using the online calculator tool (http://www.oege.org/software/hardy-weinberg.html). Multivariable Cox proportional hazard regression was used to estimate adjusted hazard ratios (HRs) and the corresponding 95% confidence intervals with respect to *CYP11B2* genotypes for the time to occurrence of the primary endpoint. We conducted a Cox proportional hazard model adjusted for age, male sex, family history of cardiovascular diseases, BMI, diabetes mellitus, dyslipidemia, and current smoking.[17] Kaplan–Meier curve estimation and log-rank tests were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered significant.

Results

Baseline characteristics of participants

Baseline characteristics of the cohort in terms of *CYP11B2* status are presented in Table 1. Among the 755 EH patients studied, 282 (37.4%) were homozygous for the T allele (TT genotype), 361 (47.8%) were heterozygous (CT genotype), and 112 (14.8%) were homozygous for the C allele (CC genotype). These frequencies were in agreement with those predicted by HWE ($p = 0.435$). No statistical differences in age or sex ratio were observed among the groups. No further significant associations with *CYP11B2* genotypes could be detected.

Primary endpoint

As shown in Table 2, after a mean follow-up period of 7.60 ±1.12 years, 27 MACEs were observed in the CC genotype group, 32 in the TT genotype group, and 56 in the CT genotype group (26.2% vs.12.4% vs. 17.1%; $p = 0.006$). CAD occurred in 19 patients of the CC genotype group, 25 of the TT genotype group, and 46 of the CT group genotype group (18.4% vs. 9.7% vs. 14.1%; $p = 0.064$). Stroke was recorded in 10 patients in the CC genotype group, 9 in the TT genotype group, and 19 in the CT genotype group (9.7% vs. 3.5% vs. 5.8%; $p = 0.061$). Two patients of the CC genotype group, one patient of the TT genotype group, and nine patients of the CT genotype group had both CAD and stroke. Three patients of the CC genotype group, one patient of the TT genotype group, and three patients of the CT genotype group died (Table 2).

As shown in Figure 1a and 1b, the time-to-event analysis using the Kaplan–Meier method showed a significantly higher rate of MACEs in the CC genotype group

Table 2. Major adverse clinical events over 11 years of follow-up according to *CYP11B2* genotype.

	CYP11B2 genotype			
	CC	CT	TT	*p*-value
MACEs, n (%)	27 (26.2)	56 (17.1)	32 (12.4)	0.006
CAD, n (%)	19 (18.4)	46 (14.1)	25 (9.7)	0.064
Stroke, n (%)	10 (9.7)	19 (5.8)	9 (3.5)	0.061
CAD + stroke, n (%)	2 (1.8)	9 (2.5)	1 (0.4)	0.080 (Fisher)
Death, n (%)	3 (2.7)	3 (0.8)	1 (0.4)	0.094 (Fisher)

MACEs, major adverse cardiovascular events; CAD, coronary artery disease.

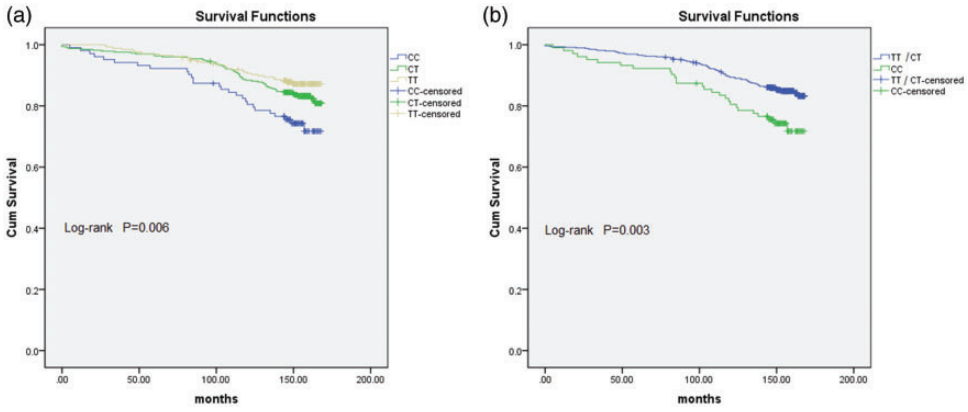

Figure 1. Kaplan–Meier curves for MACEs of subjects stratified according to genotype of the *CYP11B2* gene −344C/T polymorphism (a), and according to the CC genotype or the CT+TT genotypes (b). Differences among and between the groups were assessed using log-rank tests. A p-value < 0.05 was considered significant. The cumulative (Cum) incidence of MACEs for the CC genotype was significantly higher than that for the other genotypes. MACEs, major adverse cardiovascular events.

Table 3. Cox proportional hazard models adjusted for traditional risk factors.

	MACES		CAD		Stroke		CAD + stroke		Death	
Risk factor	HR	p	HR	p	HR	p	HR	p	HR	p
Age	1.021	0.014	1.004	0.664	1.028	0.069	1.010	0.705	1.112	0.013
Sex	0.677	0.109	0.942	0.841	0.638	0.304	3.825	0.218	0.385	0.337
Family history of hypertension	1.021	0.920	0.796	0.313	1.617	0.195	1.179	0.789	2.169	0.418
Smoking	0.516	0.016	0.644	0.179	0.689	0.424	3.792	0.216	0.753	0.777
BMI	1.005	0.857	1.030	0.345	1.056	0.279	1.158	0.062	0.762	0.084
Diabetes	1.337	0.238	1.325	0.316	0.632	0.392	0.000	0.981	1.832	0.600
Hyperlipidemia	1.156	0.514	1.021	0.932	0.991	0.981	0.541	0.325	0.822	0.842
CC genotype	2.049	0.001	1.754	0.032	2.588	0.011	1.197	0.818	2.818	0.213

MACEs, major adverse cardiovascular events; CAD, coronary artery disease; BMI, body mass index.

than in patients with the other genotypes (log rank $p = 0.006$ and $p = 0.003$ for differences among the three genotypes and between CC vs. TT+CT genotypes, respectively).

Further investigation using a Cox proportional hazard model was done to study whether the *CYP11B2* gene CC variant could predict the onset of cardiovascular diseases or mortality, independent of other risk factors. As shown in Table 3, *CYP11B2* gene CC variant was significantly and independently predictive of MACEs (HR = 2.049; $p = 0.001$) and CAD (HR = 1.754; $p = 0.032$), but not stroke, CAD plus stroke, or death.

Discussion

The present analysis is an 11-year longitudinal cohort study of Chinese EH individuals. The data demonstrated that the CC

genotype of the −344C/T *CYP11B2* polymorphism was associated with higher risk of incident cardiovascular diseases in patients with EH. This association was independent of other established cardiovascular diseases risk factors, such as age, male sex, family history of CAD and stroke, BMI, diabetes mellitus, dyslipidemia, and current smoking. Chi-square test revealed that new cases of MACEs were more frequent in the CC group than in the CT and TT groups. Kaplan–Meier curves also indicated that the CC group had an average shorter survival period.

In addition to regulating renal sodium resorption and intravascular volume, aldosterone may have direct effects on the cardiovascular system.[18,19] A cytosine/thymidine (C/T) exchange at position −344 in the promoter of *CYP11B2* affects binding of the SF-1 transcription factor,[20,21] and thus might influence gene expression. This is currently the best explanation of the association between the −344C/T polymorphism in *CYP11B2* and MACEs.

Many studies have shown a correlation between −344C/T and MACEs in different ethnicities.[22] The polymorphism was positively correlated with left ventricular mass and thickness in EH;[23,24] left ventricular hypertrophy is an important predictor of cardiovascular morbidity and mortality in patients with hypertension.[25] In a meta-analysis, Yin et al. indicated that −344C/T polymorphism might be associated with CAD in Caucasian and Asian populations.[8] However, Mishra et al.[26] indicated that the −344C/T polymorphism in *CYP11B2* is not correlated with CAD or left ventricular hypertrophy in an Indian population, although that study did not involve stratification by sex. In addition, a prospective study of healthy British men discovered that the −344C/T variant is not associated with cardiovascular risk.[27] Our current study included nearly 800 patients and showed that the −344C/T polymorphism in the *CYP11B2* gene was associated with CAD in China.

Because of genetic and environmental differences in different populations, any results cannot simply be extrapolated to a Chinese patient with hypertension. This study based on Chinese patients with EH adds to the current knowledge and reveals an association of this polymorphism with the incidence of MACEs. This polymorphism potentially influences gene expression and aldosterone levels.[5] In an in vitro study, the −344C/T polymorphism in the *CYP11B2* promoters was shown to affect binding of the SF-1 transcription factor and thus might influence gene expression; binding was 4 times stronger with the C allele than with the T allele.[21] Two studies[22,23] indicated that increased aldosterone production was associated with the C allele. Our results also demonstrated that presence of the C allele increased the risk of MACE in this Chinese EH population. Indeed, compared with traditional risk factors, such as age, sex, family history of hypertension, smoking, BMI, diabetes, hyperlipidemia, only the CC genotype significantly increased the hazard risk for MACE. Other studies have reported that the *CYP11B2* −344CC genotype was overrepresented in patients with dilated cardiomyopathy or cardiovascular diseases with extreme elevations of aldosterone.[28,29] In a study in a Tunisian Arab population, risk of CAD was associated with the −344C/T polymorphism, with interactions with age and smoking status.[30] Further studies are needed to clarify the nature and pathways of this association.

Stroke is a multifactorial and polygenic disease with major clinical manifestations and multiple etiologies. It is a significant cause of disability and death in developed countries.[31,32] The association between the aldosterone synthase −344C/T gene polymorphism and ischemic stroke remains controversial and ambiguous. A meta-analysis

of 7,710 subjects reported a lack of an association between the polymorphism and ischemic stroke.[8,17] In 2015, another meta-analysis indicated that the −344C/T variant was significantly associated with ischemic stroke, and subgroup analysis showed that it was significantly associated with ischemic stroke in East Asian and South Asian populations, but not in a Caucasian population.[30] Presently, the CYP11B2 −344C/T polymorphism could influence susceptibility to ischemic stroke. Studies have demonstrated that the frequencies of alleles are different in different ethnic populations; consequently, ischemic stroke might be affected by genetic background. Furthermore, a report indicated that this polymorphism is associated with higher aldosterone synthase activity and increased aldosterone production,[33] which in turn induces sodium and water retention, increases systemic peripheral resistance, and can lead to adverse vascular remodeling, vasoconstriction, thrombosis, and vessel wall damage, ultimately increasing the odds of stroke development.[34–36] Compared with traditional risk factors, such as sex, family history of hypertension, smoking, BMI, diabetes, hyperlipidemia, only the presence of the CC genotype had a significantly higher hazard risk.

Conclusions

In conclusion, we demonstrated that the CC variant at the −344C/T polymorphism in *CYP11B2* was significantly and independently predictive of MACEs, CAD, and stroke in this Chinese population. The −344CC genotype is implicated as a risk factor for CAD and stroke independently of other established cardiovascular risk factors in Chinese patients with EH.

Author contributions

XQ defined the research theme. LW and ZZ designed methods and carried out the laboratory experiments. DL and KY analyzed the data, interpreted the results. LW and GZ collected the clinical data from Hebei General Hospital and Beijing Xuanwu Hospital, respectively. All authors contributed to, reviewed, and approved the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Zhao Y, Yu Y, Shi M, et al. Association study to evaluate TFPI gene in CAD in Han Chinese. *BMC Cardiovasc Disord* 2017; 17: 188.
2. Bai Q, Peng B, Wu X, et al. Metabolomic study for essential hypertension patients based on dried blood spot mass spectrometry approach. *IUBMB Life* 2018; 70: 777–785.
3. Gacd Hypertension Research Programme WG, Peiris D, Thompson SR, et al. Behaviour change strategies for reducing blood pressure-related disease burden: findings from a global implementation research programme. *Implement Sci* 2015; 10: 158.
4. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; 380: 2224–2260.
5. Delles C, Erdmann J, Jacobi J, et al. Aldosterone synthase (CYP11B2) −344 C/T polymorphism is associated with left ventricular structure in human arterial hypertension. *J Am Coll Cardiol* 2001; 37: 878–884.
6. Aung M, Konoshita T, Moodley J, et al. Association of gene polymorphisms of aldosterone synthase and angiotensin converting enzyme in pre-eclamptic South African Black women. *Pregnancy Hypertens* 2018; 11: 38–43.

7. Sarhan NM, Shahin MH, El Rouby NM, et al. Effect of genetic and nongenetic factors on the clinical response to mineralocorticoid receptor antagonist therapy in Egyptians with heart failure. *Clin Transl Sci* 2020; 13: 195–203.
8. Yin C, Gu W, Gao Y, et al. Association of the -344T/C polymorphism in aldosterone synthase gene promoter with left ventricular structure in Chinese Han: A meta-analysis. *Clin Exp Hypertens* 2017; 39: 562–569.
9. Feola M, Monteverde M, Vivenza D, et al. Prognostic value of different allelic polymorphism of aldosterone synthase receptor in a congestive heart failure European continental ancestry population. *Arch Med Res* 2017; 48: 156–161.
10. Zhang H, Li X, Zhou L, et al. A novel haplotype of low-frequency variants in the aldosterone synthase gene among northern Han Chinese with essential hypertension. *Medicine (Baltimore)* 2017; 96: e8150.
11. Hoyt SB, Taylor J, London C, et al. Discovery of indazole aldosterone synthase (CYP11B2) inhibitors as potential treatments for hypertension. *Bioorg Med Chem Lett* 2017; 27: 2384–2388.
12. Kupari M, Hautanen A, Lankinen L, et al. Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. *Circulation* 1998; 97: 569–575.
13. Charoen P, Eu-Ahsunthornwattana J, Thongmung N, et al. Contribution of four polymorphisms in renin-angiotensin-aldosterone-related genes to hypertension in a Thai population. *Int J Hypertens* 2019; 2019: 4861081.
14. Sun J, Zhao M, Miao S, et al. Polymorphisms of three genes (ACE, AGT and CYP11B2) in the renin-angiotensin-aldosterone system are not associated with blood pressure salt sensitivity: A systematic meta-analysis. *Blood Press* 2016; 25: 117–122.
15. Stella P, Bigatti G, Tizzoni L, et al. Association between aldosterone synthase (CYP11B2) polymorphism and left ventricular mass in human essential hypertension. *J Am Coll Cardiol* 2004; 43: 265–270.
16. Patnaik M, Pati P, Swain SN, et al. Aldosterone synthase C-344T, angiotensin II type 1 receptor A1166C and 11-beta hydroxysteroid dehydrogenase G534A gene polymorphisms and essential hypertension in the population of Odisha, India. *J Genet* 2014; 93: 799–808.
17. Sen Z, Weida W, Jie M, et al. Coumarin glycosides from *Hydrangea paniculata* slow down the progression of diabetic nephropathy by targeting Nrf2 anti-oxidation and smad2/3-mediated profibrosis. *Phytomedicine* 2019; 57: 385–395.
18. Briet M and Schiffrin EL. Aldosterone: effects on the kidney and cardiovascular system. *Nat Rev Nephrol* 2010; 6: 261–273.
19. Funder JW. Minireview: aldosterone and the cardiovascular system: genomic and nongenomic effects. *Endocrinology* 2006; 147: 5564–5567.
20. White PC, Hautanen A and Kupari M. Aldosterone synthase (CYP11B2) polymorphisms and cardiovascular function. *J Steroid Biochem Mol Biol* 1999; 69: 409–412.
21. White PC, Hautanen A and Kupari M. Aldosterone synthase (CYP11B2) polymorphisms and cardiovascular function. *Endocr Res* 1998; 24: 797–804.
22. Liu Y, Liu HL, Han W, et al. Association between the CYP11B2 gene -344T>C polymorphism and coronary artery disease: a meta-analysis. *Genet Mol Res* 2015; 14: 3121–3128.
23. Wilson PW. Established risk factors and coronary artery disease: the Framingham Study. *Am J Hypertens* 1994; 7: 7S–12S.
24. Lawes CM, Vander Hoorn S, Rodgers A, et al. Global burden of blood-pressure-related disease, 2001. *Lancet* 2008; 371: 1513–1518.
25. Bang CN, Soliman EZ, Simpson LM, et al. Electrocardiographic left ventricular hypertrophy predicts cardiovascular morbidity and mortality in hypertensive patients: The ALLHAT Study. *Am J Hypertens* 2017; 30: 914–922.
26. Mishra A, Srivastava A, Mittal T, et al. Impact of renin-angiotensin-aldosterone system gene polymorphisms on left

ventricular dysfunction in coronary artery disease patients. *Dis Markers* 2012; 32: 33–41.
27. Payne JR, Dhamrait SS, Toor IS, et al. The −344T>C promoter variant of the gene for aldosterone synthase (CYP11B2) is not associated with cardiovascular risk in a prospective study of UK healthy men. *Atherosclerosis* 2004; 174: 81–86.
28. Bress A, Han J, Patel SR, et al. Association of aldosterone synthase polymorphism (CYP11B2 -344T>C) and genetic ancestry with atrial fibrillation and serum aldosterone in African Americans with heart failure. *PLoS One* 2013; 8: e71268.
29. Takai E, Akita H, Kanazawa K, et al. Association between aldosterone synthase (CYP11B2) gene polymorphism and left ventricular volume in patients with dilated cardiomyopathy. *Heart* 2002; 88: 649–650.
30. Yu Y. The CYP11B2 -344C/T variant is associated with ischemic stroke risk: An updated meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2015; 16: 382–388.
31. Pi Y, Zhang L, Yang Q, et al. Apolipoprotein A5 gene promoter region-1131T/C polymorphism is associated with risk of ischemic stroke and elevated triglyceride levels: a meta-analysis. *Cerebrovasc Dis* 2012; 33: 558–565.
32. Hankey GJ. Potential new risk factors for ischemic stroke: what is their potential? *Stroke* 2006; 37: 2181–2188.
33. LeHoux JG, Dupuis G and Lefebvre A. Control of CYP11B2 gene expression through differential regulation of its promoter by atypical and conventional protein kinase C isoforms. *J Biol Chem* 2001; 276: 8021–8028.
34. Saidi S, Mahjoub T and Almawi WY. Aldosterone synthase gene (CYP11B2) promoter polymorphism as a risk factor for ischaemic stroke in Tunisian Arabs. *J Renin Angiotensin Aldosterone Syst* 2010; 11: 180–186.
35. Osmond JM, Rigsby CS and Dorrance AM. Is the mineralocorticoid receptor a potential target for stroke prevention? *Clin Sci (Lond)* 2008; 114: 37–47.
36. Safar ME, Cattan V, Lacolley P, et al. Aldosterone synthase gene polymorphism, stroke volume and age-related changes in aortic pulse wave velocity in subjects with hypertension. *J Hypertens* 2005; 23: 1159–1166.