Association of *CYP11B2* polymorphisms with metabolic syndrome patients

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Abstract. Aldosterone synthase is a key enzyme in aldosterone production. Polymorphisms of the aldosterone synthase gene, *CYP11B2*, have been suggested to be involved in the pathogenesis of diabetes mellitus (DM), hypertension and cardiovascular diseases. In the light of these findings, we hypothesized that *CYP11B2* genetic polymorphisms play a role in metabolic syndrome (MetS). Therefore, we investigated the associations of three *CYP11B2* polymorphisms [-344T>C, K173R and intron 2 conversion (IC)] with Korean MetS patients. In total, 640 subjects comprising 320 cases and 320 control individuals) were included in the present study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques were used to assess *CYP11B2* polymorphisms. The *CYP11B2* -344T>C, K173R and IC polymorphisms did not exhibit a significant difference in the genotype and allele frequencies between the MetS and control groups. However, the -344T>C polymorphism in males and haplotypes comprising the three polymorphisms were associated with susceptibility to MetS. Thus, the pattern of haplotype associations was gender-specific. Based on these results, the -344T>C polymorphism in males and haplotypes of the *CYP11B2* gene potentially affect MetS susceptibility. These findings remain to be confirmed in various ethnic populations with a larger sample size.

Introduction

Aldosterone synthase plays an important role in determining the levels of aldosterone secretion as one of the main effectors of the renin-angiotensin-aldosterone system (RAAS). Polymorphisms of the aldosterone synthase gene, *CYP11B2*, have attracted attention in the development of diabetes, hypertension and cardiovascular diseases.

The aldosterone synthase gene (*CYP11B2*) is assigned to chromosome 8 near the highly homologous *CYP11B1* encoding 11-β-hydroxylase (1). Several frequent polymorphisms have been reported in the *CYP11B2* gene. These polymorphisms are suggested to be associated with serum aldosterone levels (2), hyperaldosteronism (3), plasma glucose levels and glucose intolerance (4), type II diabetes mellitus (DM) (5), left ventricular size and mass (6,7), arterial stiffness (8), myocardial infarction (9,10) and hypertension (11-14), although these associations remain to be clarified.

High blood pressure, abnormal levels of lipid and glucose are the main risk components of metabolic syndrome (MetS), which is a highly prevalent disorder worldwide. Therefore, we hypothesized that the *CYP11B2* genetic polymorphism plays a role in MetS development. Nevertheless, the associations of *CYP11B2* polymorphisms and risk assessment of MetS have not been studied extensively.

Thus, we investigated associations of the *CYP11B2* polymorphisms of -344T>C (rs1799998), K173R (rs4539) and intron 2 conversion (IC) with MetS patients in a Korean population.

Materials and methods

Study population. A total of 320 MetS patients [mean age ± standard deviation (SD), 50.94±8.434] and 320 healthy control subjects (mean age ± SD, 49.86±11.76 years) were included in this study. We used the National Cholesterol Education Program Adult Treatment Panel III definition for MetS (15) with slight modifications, including waist circumference (WC) (≥90 cm in men, ≥85 cm in women); increased triglycerides level (≥150 mg/dl); high-density lipoprotein (HDL)-cholesterol (<40 mg/dl in men, <50 mg/dl in women); increased blood pressure (≥130/85 mmHg or antihypertensive medication) and increased fasting blood glucose (≥100 mg/dl or type II DM). Individuals with ≥3 traits of the risk factors described above were defined as MetS patients. The control group comprised healthy individuals without a history of hypertension, DM, cardiovascular or other diseases to exclude those with MetS.

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Key words: CYP11B2, metabolic syndrome, polymorphism

Table I. Comparison of demographic characteristics between MetS patients and controls.

Characteristics	Control	MetS patients	P[a]
No.	320	320	
Age, years (mean ± SD)	50.94±8.434	49.86±11.76	0.183
BMI (mean ± SD)	23.87±2.715	27.40±2.646	<0.001
FBS (mean ± SD)	87.35±10.09	104.41±26.67	<0.001
TG (mean ± SD)	86.53±39.57	211.89±170.81	<0.001
HDL-cholesterol (mean ± SD)	57.01±13.57	44.44±10.41	<0.001
SBP (mean ± SD)	117.99±12.03	133.61±14.53	<0.001
DBP (mean ± SD)	70.84±8.531	81.95±10.43	<0.001
WC (mean ± SD)	72.07±9.159	85.38±10.28	<0.001

[a]Chi-square test for categorical data, two-sided t-test for continuous data. MetS, metabolic syndrome; SD, standard deviation; BMI, body mass index; FBS, fasting blood sugar; TG, triglyceride; HDL-cholesterol, high-density lipoprotein-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference.

The biospecimen and data used in this study werew provided by the Jeju National University Hospital Biobank, a member of the National Biobank of South Korea, which is supported by the Ministry of Health and Welfare. All samples obtained were from Korean individuals and were collected together with written informed consent according to protocol approved by the Institutional Review Board of the Jeju National University Hospital in June 2002.

Polymorphism analysis. Total genomic DNA was extracted according to the manufacturer's instructions (Qiagen, Inc., Valencia, CA, USA). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques were used to detect *CYP11B2* polymorphisms with slight modifications of the method used by Gu *et al* (12). The PCR for the -344T/C polymorphism was performed using sense (5'-GTG TCA GGG CAG GGG GTA-3') and antisense (5'-AGG CGT GGG GTC TGG ACT-3') primers. DNA was amplified for 35 cycles with denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 1 min. The amplified PCR products were 228 bp and digested with a restriction endonuclease, *Hae*III (New England Biolabs, Beverly, MA, USA) at 37˚C for 17 h. The primer sequences used to detect the K173R polymorphism were 5'-GAA AAG GCT GCA GCT CGA ACA CAA-3' (sense) and 5'-GCA TGG CCC ACA CCT TCT A-3' (antisense). DNA was amplified for 35 cycles with denaturation at 94˚C for 30 sec, annealing at 65˚C for 30 sec and extension at 72˚C for 1 min. Bands of 371 bp were amplified by PCR and digested with a restriction endonuclease *Bsu*36I (New England Biolabs). The IC polymorphism was analyzed using two separate PCRs, one to amplify the wild-type gene (W) and another to amplify the conversion gene (C). The forward primer sequence for the wild-type gene was 5'-TGG AGA AAA GCC CTA CCC TGT-3', whereas 5'-CAG AAA ATC CCT CCC CCC TA-3' was used for the conversion gene. The same reverse primer: 5'-AGG AAC CTC TGC ACG GCC-3' was used for the two PCRs. PCR conditions were run for 35 cycles with denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 1 min. The size of PCR products in each reaction was ~418 bp and was directly detected by 2% agarose gel electrophoresis.

Measurements of clinical and anthropometric traits. For measurements of biochemical traits, venous blood of the study subjects was collected into EDTA tubes after overnight fasting. The levels of plasma glucose, triglycerides and HDL-cholesterol were measured enzymatically on an automatic analyzer (TBA 200FR NEO; Toshiba Medical Systems, Tokyo, Japan).

Anthropometric traits containing body mass index (BMI) and WC were measured. BMI was calculated as weight (kg) divided by height (m^2). Using a flexible tape, WC was measured at the smallest horizontal circumference between the costal margin and iliac crest. Measurements of blood pressures were obtained on the right arm at a seated position after 10 min of rest.

Statistical analysis. Data were expressed as means ± SD. Clinical characteristics were compared by the Student's unpaired t-test. The χ^2 test was used to compare genotype and allele frequencies of *CYP11B2* polymorphisms between the cases and controls and to test for Hardy-Weinberg equilibrium. Associations of the studied polymorphisms with MetS prevalence were calculated using adjusted odds ratios (AORs) and 95% confidence intervals (95% CIs) from multivariate logistic regression adjusted for age and gender. StatsDirect statistical software, version 2.4.4 (StatsDirect, Ltd., Altrincham, UK) was used to calculate the AOR and 95% CIs. P<0.05 was considered to indicate statistically significant differences. Haplotypes for multiple loci were estimated using the expectation-maximization algorithm with SNPAlyze (version 5.1; Dynacom Co., Ltd., Yokohama, Japan). The linkage disequilibrium (LD) between loci was measured using the absolute value of Lewontin's D (16).

Results

Comparison of patient characteristics. MetS patients had significantly higher values for MetS risk parameters such as

Table II. The genotype and allele frequencies of the *CYP11B2* -344T>C, K173R and IC polymorphisms between control subjects and patients with MetS.

Genotype	Controls (n=320)	MetS (n=320)	AOR (95% CI)	P[a]
CYP11B2 -344T>C				
TT	157 (49.1)	157 (49.1)	1.000 (Reference)	
TC	138 (43.1)	145 (45.3)	1.087 (0.771-1.532)	0.634
CC	25 (7.8)	18 (5.6)	0.630 (0.322-1.230)	0.176
Dominant (TT vs. TC+CC)			1.005 (0.723-1.396)	0.979
Recessive (TT+TC vs. CC)			0.583 (0.302-1.126)	0.108
T allele	452 (70.6)	459 (71.7)	1.000 (Reference)	
C allele	188 (29.4)	181 (28.3)	0.923(0.714-1.193)	0.539
CYP11B2 K173R				
KK	164 (51.3)	148 (46.2)	1.000 (Reference)	
KR	136 (42.5)	156 (48.8)	1.261 (0.897-1.774)	0.183
RR	20 (6.2)	16 (5.0)	0.761 (0.368-1.576)	0.462
Dominant (KK vs. KR+RR)		1.196 (0.859-1.665)	0.288	
Recessive (KK+KR vs. RR)		0.661 (0.325-1.346)	0.254	
CYP11B2 IC				
K allele	464 (72.5)	452 (70.6)	1.000 (Reference)	
R allele	176 (27.5)	188 (29.4)	1.056 (0.816-1.367)	0.680
WW	215 (67.2)	232 (72.5)	1.000 (Reference)	
WC	90 (28.1)	75 (23.4)	0.821 (0.561-1.202)	0.310
CC	15 (4.7)	13 (4.1)	0.712 (0.319-1.585)	0.405
Dominant (WW vs. WC+CC)			0.804 (0.562-1.151)	0.234
Recessive (WW+WC vs. CC)			0.743 (0.335-1.651)	0.466
W allele	520 (81.3)	539 (84.2)	1.000 (Reference)	
C allele	120 (18.7)	101 (15.8)	0.815 (0.599-1.109)	0.1929

[a]Adjusted by age and gender. IC, intron 2 conversion; MetS, metabolic syndrome; AOR, adjusted odds ratio; 95% CI, 95% confidence interval; W, wild-type gene; C, conversion gene.

BMI, glucose, blood pressure, triglycerides and WC (P<0.0001), but lower levels of HDL-cholesterol (P<0.0001) than the control subjects (Table I).

Genotype and allele frequencies of the polymorphisms for MetS and control subjects. A comparison was made of the genotype and allele frequencies of the *CYP11B2* -344T>C, K173R and IC polymorphisms between the MetS and control groups (Table II). The genotype frequencies for all of the polymorphisms were in accordance with the Hardy-Weinberg equilibrium in the two groups. The genotype and allele frequencies of the -344T>C, K173R and IC polymorphisms were not significantly different between the MetS and control groups. However, when the data were stratified by gender, the recessive (TT+TC vs. CC) distribution of the -344T>C polymorphism showed a significant association (OR=0.438, 95% CI: 0.208-0.922, P=0.030) with increased MetS risk only in the male group (Table III).

Haplotype frequencies of the polymorphisms for MetS and control subjects. To expand the available association data, we evaluated the effectiveness of allelic combinations using haplotypes of *CYP11B2* three polymorphic loci (-344T>C, K173R and IC) (Table IV). Haplotype analysis revealed that the C-R-W haplotype was significantly more frequent in the MetS patients than in the control subjects, suggesting an association with increased MetS susceptibility (OR=1.412, 95% CI: 1.075-1.856, P=0.016). In addition, the T-R-W, C-K-W and C-R-C haplotype showed a significantly lower association with the risk of MetS.

Haplotype frequencies of the polymorphisms according to gender. When the haplotype data were stratified by gender, the patterns of association with MetS were found to be gender-specific (Table V). The C-R-W haplotype was significantly associated with MetS susceptibility (OR=1.429, 95% CI: 1.006-2.031, P=0.047) in men only as compared to the total number of subjects. By contrast, the C-K-W haplotype in men and the T-K-C haplotype in women was significantly associated with a lower risk of MetS.

D prime (D') was calculated to assess the level of LD between the K173R and -344T>C polymorphisms in the controls plus patients. Polymorphisms *CYP11B2*, K173R and -344T>C were in moderate level of LD (D'=0.67).

Table III. The genotype and allele frequencies of the *CYP11B2* -344T>C, K173R and IC polymorphisms between the control subjects and MetS patients in men.

Genotype	Controls (n=152)	MetS (n=254)	AOR (95% CI)	P[a]
CYP11B2 -344T/C				
TT	78 (51.3)	122 (48.0)	1.000 (Reference)	
TC	57 (37.5)	118 (46.5)	1.293 (0.842-1.986)	0.240
CC	17 (11.2)	14 (5.5)	0.480 (0.221-1.042)	0.064
Dominant (TT vs. TC+CC)			1.108 (0.738-1.663)	0.621
Recessive (TT+TC vs. CC)			0.438 (0.208-0.922)	0.030
T allele	213 (70.1)	362 (71.3)	1.000 (Reference)	
C allele	91 (29.9)	146 (28.7)	0.918 (0.670-1.258)	0.595
CYP11B2 K173R				
KK	79 (52.0)	115 (45.3)	1.000 (Reference)	
KR	61 (40.1)	125 (49.2)	1.319 (0.862-2.019)	0.202
RR	12 (7.9)	14 (5.5)	0.734 (0.319-1.688)	0.466
Dominant (KK vs. KR+RR)			1.228 (0.816-1.848)	0.326
Recessive (KK+KR vs. RR)			0.629 (0.281-1.410)	0.260
K allele	219 (72.0)	355 (69.9)	1.000 (Reference)	
R allele	85 (28.0)	153 (30.1)	1.055 (0.768-1.450)	0.741
CYP11B2 IC				
WW	105 (69.1)	184 (72.5)	1.000 (Reference)	
WC	38 (25.0)	59 (23.2)	0.933 (0.578-1.507)	0.778
CC	9 (5.9)	11 (4.3)	0.686 (0.273-1.721)	0.422
Dominant (WW vs. WC+CC)			0.886 (0.567-1.382)	0.593
Recessive (WW+WC vs. CC)			0.699 (0.281-1.738)	0.441
W allele	248 (81.6)	427 (84.1)	1.000 (Reference)	
C allele	56 (18.4)	81 (15.9)	0.862 (0.590-1.258)	0.440

[a]Adjusted by age and gender. IC, intron 2 conversion; MetS, metabolic syndrome; AOR, adjusted odds ratio; 95% CI, 95% confidence interval; W, wild-type gene; C, conversion gene.

Table IV. Haplotype frequencies of *CYP11B2* -344T>C, K173R and IC polymorphisms between patients with MetS and controls.

Haplotype	Controls (2n=640, %)	MetS (2n=640, %)	OR (95% CI)	P[a]
CYP11B2 -344T>C/K173R/IC				
T-K-W	309 (48.3)	340 (53.1)	1.214 (0.975-1.512)	0.094
T-K-C	93 (14.5)	85 (13.2)	0.901 (0.656-1.237)	0.572
T-R-W	47 (7.4)	29 (4.5)	0.599 (0.372-0.964)	0.044
T-R-C	3 (0.5)	6 (0.9)	2.009 (0.500-8.073)	0.506
C-K-W	50 (7.9)	21 (3.2)	0.400 (0.238-0.675)	0.001
C-K-C	12 (1.9)	7 (1.1)	0.579 (0.226-1.480)	0.356
C-R-W	114 (17.8)	150 (23.4)	1.412 (1.075-1.856)	0.016
C-R-C	12 (1.9)	3 (0.5)	0.247 (0.069-0.878)	0.034

[a]Fisher's exact test. IC, intron 2 conversion; MetS, metabolic syndrome; OR; odds ratio, 95% CI, 95% confidence interval; W, wild-type gene; C, conversion gene.

Discussion

CYP11B2, a component of the RAAS, plays an important role in determining the levels of aldosterone secretion. Its transcripts levels are low in normal adrenals, but are significantly increased in aldosterone-producing adenomas (17).

Although the results of *CYP11B2* polymorphisms are conflicting among various populations, previous studies have

Table V. Haplotype frequencies of the *CYP11B2* polymorphisms according to gender.

	Male				Female			
Haplotype	Controls (2n=304)	MetS (2n=508)	OR (95% CI)	P[a]	Controls (2n=336)	MetS (2n=132)	OR (95% CI)	P[a]
CYP11B2 -344T>C/K173R/IC								
T-K-W	148 (48.7)	268 (52.8)	1.177 (0.886-1.564)	0.277	161 (47.8)	77 (58.5)	1.522 (1.013-2.286)	0.051
T-K-C	42 (13.7)	69 (13.5)	0.981 (0.649-1.482)	0.916	51 (15.3)	10 (7.5)	0.458 (0.225-0.932)	0.032
T-R-W	22 (7.1)	21 (4.2)	0.553 (0.299-1.023)	0.074	26 (7.8)	7 (5.4)	0.668 (0.283-1.578)	0.426
T-R-C	2 (0.5)	4 (0.7)	1.198 (0.218-6.585)	1.000	1 (0.3)	3 (2.2)	7.791 (0.803-75.62)	0.070
C-K-W	22 (7.2)	12 (2.3)	0.310 (0.151-0.636)	0.002	29 (8.5)	9 (6.7)	0.775 (0.356-1.684)	0.578
C-K-C	8 (2.5)	6 (1.3)	0.442 (0.152-1.287)	0.163	4 (1.3)	1 (0.8)	0.634 (0.070-5.725)	1.000
C-R-W	57 (18.6)	126 (24.8)	1.429 (1.006-2.031)	0.047	57 (16.9)	19 (14.4)	0.823 (0.469-1.446)	0.578
C-R-C	5 (1.7)	2 (0.5)	0.236 (0.046-1.226)	0.110	7 (2.2)	6 (4.7)	2.238 (0.738-6.790)	0.207

[a]Fisher's exact test. MetS, metabolic syndrome; OR, odds ratio; 95% CI, 95% confidence interval; IC, intron 2 conversion; W, wild-type gene; C, conversion gene.

shown that several *CYP11B2* polymorphisms are associated with its protein production (2,3). Human vascular diseases such as left ventricular size and mass (6,7), arterial stiffness (8), myocardial infarction (9,10) and hypertension (11-14) are associated with *CYP11B2* polymorphisms. Plasma glucose levels and glucose intolerance (4) and type II diabetes (5) have also been associated with *CYP11B2* polymorphisms, suggesting the involvement of CYP11B2 in MetS development. These observations lead to the hypothesis that the *CYP11B2* gene is associated with MetS. Therefore, in view of the importance of CYP11B2, we determined whether there is an association of *CYP11B2* -344C>T, K173R and IC polymorphisms with Korean MetS patients.

In the present study, the -344T/C, K173R and IC polymorphisms of the *CYP11B2* did not exhibit significant differences between the MetS and control groups. The recessive (TT+TC vs. CC) distribution of the -344T>C polymorphism and haplotypes of the *CYP11B2* gene were, however, associated with susceptibility of MetS patients. At present, few studies have investigated the correlation of *CYP11B2* polymorphisms to MetS, with studies mainly focusing on hypertension. Russo *et al* (18) have reported that the -344C>T polymorphism of the *CYP11B2* is associated with MetS in European populations including Italy, the United Kingdom and Belgium. The C allele of the variant was associated with MetS in men but not in women. Although our data were not associated with the C allele, a significant association with MetS was observed only in the recessive (TT+TC vs. CC) genotype of the men included in the study. By contrast, Bellili *et al* (5) did not find any association of the -344T>C polymorphism with MetS in a French population. Therefore, the results of the -344T>C polymorphism on MetS remain inconclusive. In previous studies (19-22), the -344T>C and K173R polymorphisms were found strong in LD. Polymorphisms *CYP11B2*, K173R and -344T>C of this study exhibited a moderate level of LD. Therefore, further prospective and cross-sectional studies with larger sample sizes of various populations are required to evaluate the exact role of *CYP11B2* -344T>C polymorphism in MetS patients.

As the results of the haplotype analysis from three diallelic RFLPs (-344T>C, K173R and IC) of this study reveal, the C-R-W haplotype was associated with higher MetS susceptibility in the total number of subjects and men. By contrast, the T-R-W, C-K-W and C-R-C haplotypes of the total number of subjects, the C-K-W haplotype in men and the T-K-C haplotype in women showed a significantly lower association with the risk of MetS. Therefore these haplotypes may be in LD with other functionally important polymorphisms in *CYP11B2* or *CYP11B1*. However, Bellili *et al* (5) studied the -344T/C and 3097G>A polymorphisms of *CYP11B2* gene in a French MetS population. The AA genotype of the 3097G>A polymorphism was associated with a lower incidence of the MetS in men but not in women. The -344T>C polymorphism was, however, not associated with MetS. In the haplotype analysis from the -344T>C and 3097G>A polymorphisms, the T-A and C-A haplotypes were associated with a lower incidence of the total MetS and men MetS, respectively. From these results, haplotype analysis using multiple polymorphic loci than single nucleotide polymorphism may be beneficial in defining more specific genotypes associated with MetS risk.

MetS is defined by a cluster of factors such as obesity, lipid metabolic disorders, diabetes and hypertension. However, the exact definition and pathophysiology of MetS remains to be elucidated. MetS is a multifactorial disorder in which still largely unknown genetic determinants and environmental factors, including lifestyle and dietary habits, are involved. Therefore more genetic markers should be identified for MetS.

In conclusion, although *CYP11B2* polymorphisms studied in this study were not associated with an increased risk of MetS, the -344T>C polymorphism in men and haplotypes were associated with MetS susceptibility. Based on these results, the associations between *CYP11B2* polymorphisms and patients with MetS in various populations using a larger sample size remain to be verified.

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References

1. Taymans SE, Pack S, Pak E, Torpy DJ, Zhuang Z and Stratakis CA: Human CYP11B2 (aldosterone synthase) maps to chromosome 8q24.3. J Clin Endocrinol Metab 83: 1033-1036, 1998.
2. Russo P, Siani A, Venezia A, Iacone R, Russo O, Barba G, D'Elia L, Cappuccio FP and Strazzullo P: Interaction between the C(-344)T polymorphism of CYP11B2 and age in the regulation of blood pressure and plasma aldosterone levels: cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. J Hypertens 20: 1785-1792, 2002.
3. Zhang GX, Wang BJ, Ouyang JZ, Deng XY, Ma X, Li HZ, Wu Z, Liu SL, Xu H and Zhang X: Polymorphisms in CYP11B2 and CYP11B1 genes associated with primary hyperaldosteronism. Hyperten Res 33: 478-484, 2010.
4. Ranade K, Wu KD, Risch N, Olivier M, Pei D, Hsiao CF, Chuang LM, Ho LT, Jorgenson E, Pesich R, *et al*: Genetic variation in aldosterone synthase predicts plasma glucose levels. Proc Natl Acad Sci USA 98: 13219-13224, 2001.
5. Bellili NM, Foucan L, Fumeron F, Mohammedi K, Travert F, Roussel R, Balkau B, Tichet J and Marre M: Associations of the -344 T>C and the 3097 G>A polymorphisms of CYP11B2 gene with hypertension, type 2 diabetes, and metabolic syndrome in a French population. Am J Hypertens 23: 660-667, 2010.
6. Kupari M, Hautanen A, Lankinen L, Koskinen P, Virolainen J, Nikkila H and White PC: Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. Circulation 97: 569-575, 1998.
7. Sookoian S, Gianotti TF and Pirola CJ: Role of the C-344T aldosterone synthase gene variant in left ventricular mass and left ventricular structure-related phenotypes. Heart 94: 903-910, 2008.
8. Pojoga L, Gautier S, Blanc H, Guyene TT, Poirier O, Cambien F and Benetos A: Genetic determination of plasma aldosterone levels in essential hypertension. Am J Hypertens 11: 856-860, 1998.
9. White PC, Hautanen A and Kupari M: Aldesterone synthase (CYP11B2) polymorphisms and cardiovascular function. Endocr Res 24: 797-804, 1998.
10. Hautanen A, Toivanen P, Manttari M, Tenkanen L, Kupari M, Manninen V, Kayes KM, Rosenfeld S and White PC: Joint effects of an aldosterone synthase (CYP11B2) gene polymorphism and classic risk factors on risk of myocardial infarction. Circulation 100: 2213-2218, 1999.
11. Tsukada K, Ishimitsu T, Teranishi M, Saitoh M, Yoshii M, Inada H, Ohta S, Akashi M, Minami J, Ono H, *et al*: Positive association of CYP11B2 gene polymorphism with genetic predisposition to essential hypertension. J Hum Hypertens 16: 789-793, 2002.
12. Gu D, Ge D, He J, Li B, Chen J, Liu D, Chen J and Chen R: Haplotypic analyses of the aldosterone synthase gene CYP11B2 associated with stage-2 hypertension in northern Han Chinese. Clin Genet 66: 409-416, 2004.
13. Komiya I, Yamada T, Takara M, Asawa T, Shimabukuro M, Nishimori T and Takasu N: Lys(173)Arg and -344T/C variants of CYP11B2 in Japanese patients with low-renin hypertension. Hypertension 35: 699-703, 2000.
14. Lim PO, Macdonald TM, Holloway C, Friel E, Anderson NH, Dow E, Jung RT, Davies E, Fraser R and Connell JM: Variation at the aldosterone synthase (CYP11B2) locus contributes to hypertension in subjects with a raised aldosterone-to-renin ratio. J Clin Endocrinol Metab 87: 4398-4402, 2002.
15. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, *et al*: Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112: 2735-2752, 2005.
16. Hedrick PW: Gametic disequilibrium measures: proceed with caution. Genetics 117: 331-341, 1987.
17. Shibata H, Suzuki H, Ogishima T, Ishimura Y and Saruta T: Significance of steroidogenic enzymes in the pathogenesis of adrenal tumour. Acta Endocrinol (Copenh) 128: 235-242, 1993.
18. Russo P, Lauria F, Loguercio M, Barba G, Arnout J, Cappuccio FP, de Lorgeril M, Donati MB, Iacoviello L, Krogh V, *et al*: -344C/T variant in the promoter of the aldosterone synthase (CYP11B2) is associated with metabolic syndrome in men. Am J Hypertens 20: 218-222, 2007.
19. Imrie H, Freel M, Mayosi BM, Davies E, Fraser R, Ingram M, Cordell HJ, Farrall M, Avery PJ, Watkins H, *et al*: Association between aldosterone production and variation in the 11beta-hydroxylase (CYP11B1) gene. J Clin Endocrionol Metab 91: 5051-5056, 2006.
20. Rajput C, Arif E, Vibhuti A, Stobdan T, Khan AP, Norboo T, Afrin F and Qadar Pasha MA: Predominance of interaction among wild-type alleles of CYP11B2 in Himalayan natives associates with high-altitude adaption. Biochem Biophys Res Commun 348: 735-740, 2006.
21. Qi Y, Niu W, Zhu T, Zhou W and Qiu C: Synergistic effect of the genetic polymorphisms of the renin-angiotensin-aldosterone system on high-altitude pulmonary edema: a study from Qinghai-Tibet altitude. Eur J Epidemiol 23: 143-152, 2008.
22. Chen B, Nie S, Luo S, Zhang W and Xiao C: Association of the human CYP11B2 gene and essential hypertension in southwest Han Chinese population: a haplotype-based case-control study. Clin Exp Hypertens 33: 106-112, 2011.