RESEARCH ARTICLE

Aldosterone synthase C-344T, angiotensin II type 1 receptor A1166C and 11-β hydroxysteroid dehydrogenase G534A gene polymorphisms and essential hypertension in the population of Odisha, India

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Abstract

Essential hypertension which accounts 90–95% of the total hypertension cases is affected by both genetic and environmental factors. This study was undertaken to investigate the association of aldosterone synthase C-344T, angiotensin II type I receptor A1166C and 11-β hydroxysteroid dehydrogenase type 2 G534A polymorphisms with essential hypertension in the population of Odisha, India. A total of 246 hypertensive subjects (males, 159; females, 87) and 274 normal healthy individuals (males, 158; females, 116) were enrolled in this study based on the inclusion and exclusion criteria. Analysis of genetic and biochemical data revealed that in this population the CT and TT genotypes of aldosterone synthase C-344T polymorphism, frequency of alcohol consumption and aldosterone levels were significantly high among the total as well as male hypertensives, while the AC and CC genotypes of angiotensin II type I receptor A1166C polymorphism were significantly high among the total as well as female hypertensives. High density lipoprotein levels were higher in male hypertensives.

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Introduction

Essential hypertension is a complex multifactorial condition influenced by both genetic and environmental factors (Lifton *et al.* 2001). However, renin–angiotensin–aldosterone system (RAAS) and other factors that influence renal sodium handling through regulation of secretion and/or action of aldosterone have been observed to be the strong contributors to the development of hypertension (Strazzullo *et al.* 2003). Therefore, polymorphisms of RAAS genes have been under intense investigation to determine their association with clinical expression of essential hypertension.

The aldosterone synthase gene (*CYP11B2*), encodes for a cytochrome P450 enzyme, involved in the terminal steps of aldosterone synthesis in the zona glomerulosa cells of human adrenal glands (Clyne *et al.* 1997). Aldosterone mediates sodium balance and arterial pressure by influencing intravascular volume and arterial thickness (Freel and Connel 2004). Though several polymorphisms have been identified in *CYP11B2* gene, −344 (C→T) mutation in promoter region that upregulates aldosterone production has been reported to be associated with essential hypertension in different population groups with contrasting results (Tsukada *et al.* 2002; Hu *et al.* 2006; Rajan *et al.* 2010).

Of the two subtypes of angiotensin II receptors (angiotensin II type I and type II receptors), the type I receptor appears to mediate most of the known physiological and pathological effects of angiotensin II including vascular contraction, pressure responses, renal tubular sodium transport and aldosterone secretion, those known to play key roles in blood pressure regulation. Consequently, polymorphisms in the angiotensin II type I receptor (*ATR1)* gene have the potential to affect clinical expression of high blood

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pressure. Though genetic association studies have shown inconclusive results yet A→ C transversion at position 1166 in the 3′-untranslated region of *ATR1* gene has been observed to influence the clinical expression of essential hypertension (Bonnardeaux *et al.* 1994; Castellano *et al.* 1996; Schmidt *et al.* 1997; Szombathy *et al.* 1998).

Aldosterone, expressed in kidney and colon, acts on the target cells by binding to the mineralocorticoid receptor (MR) with an affinity similar to cortisol. However, 11-β hydroxysteroid dehydrogenase type II enzyme acts as a gatekeeper and prevents nonspecific activation of the receptor by higher levels of circulating cortisol (100–1000 times compared to aldosterone) in the circulation by converting it into inactive cortisone, which shows no affinity for MR and thus defending the mineralocorticoid receptor from being overwhelmed by glucocorticoids (Funder *et al.* 1988). Mutations in 11-β hydroxysteroid dehydrogenase type 2 (*11βHSD2*) gene generating compromised enzyme activity leads to an overstimulation of the MR by cortisol, thus causing sodium retention, hypokalemia and high blood pressure (Odermatt *et al.* 2001). To date around 20 different mutations have been reported in exons 3–5 of the *11βHSD2* gene that results in functional impairment of the gene. But, G→A substitution at position 534 in exon 3 of the *11βHSD2* gene has been studied extensively in context to development of essential hypertension with variable findings (Melander *et al.* 2000; Mariniello *et al.* 2005; Campino *et al.* 2012).

Interethnic differences in vascular disease demography indicate the need to examine the relationship between genetic polymorphisms and hypertension in different population groups across the globe to establish a genetic basis of the pathogenesis of vascular diseases. The three genes, *CYP11B2*, *ATR1* and *11βHSD2* have been studied extensively in Caucasians and Orientals with variable results. Since no data is available, we made an attempt to find out the distribution of genetic polymorphisms of the these three genes and their relationship to essential hypertension in the population of Odisha, an eastern Indian state. This will provide additional information to the existing global data bank.

Materials and methods

Odisha state is situated in the eastern part of India along the Bay of Bengal. The total population of the state is 42.5 million (size growth rate and distribution of population, http://www.censusindia.gov.in). Of them, 22.01% belong to tribal (aboriginal) communities and the rest belong to general communities mostly of Indo-Aryan origin.

Subject selection

The subjects were selected from Capital Hospital, Bhubaneswar, the capital city of the state and VSS Medical College and Hospital, Burla, about 300 km from Bhubaneswar towards the north west direction, from January 2011 to August 2012. A total of 246 hypertensive (patients) and 274 normotensive (controls) individuals were enrolled for this study. All the subjects belonged to the general community and were of Indo-Aryan in origin. The inclusion criteria for patients were systolic blood pressure (SBP) ≥ 140 mm Hg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg (18) or with prescribed antihypertensive medication. The exclusion criteria were individuals with secondary hypertension (hypertension due to renovascular disease, renal failure, pheochromocytoma, aldosteronism or other causes of secondary hypertension), diabetes or under any kind of lipid-lowering drugs/statins which was confirmed by medical evaluation by the consultant doctor. The controls did not show history of hypertension, diabetes or any other cardiovascular diseases and were not under any lipid-lowering drug. Informed consent was obtained from all individuals before enrollment. This study was approved by the Institutional Ethical Committee.

Data collection

Blood pressure was measured by the treating physician using a sphygmomanometer in sitting position from the right arm (Pickering *et al.* 2005). Data on age, sex, family history, habits of tobacco and alcohol consumption were recorded. Extobacco users and alcoholics were excluded from the study. Body mass index (BMI) was calculated using the formula (weight in kg) / (height in meters)^2 and individuals with BMI ≥23 kg/m^2 and ≥25 kg/m^2 were classified as overweight and obese, respectively (WHO 2000).

Blood sample collection

At least 2 mL of venous blood was collected from the subjects after overnight fasting and stored in EDTA vials. From 1 mL of the collected blood, plasma was separated within 3 h by centrifugation at 3000 rpm for 3 min and stored at −20°C for biochemical analysis. Blood/plasma samples were transferred to the laboratory under cold conditions for detailed investigation.

Biochemical analysis

Lipid profiling (includes total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides) and kidney function tests (urea and creatinine) were carried out with an automatic analyzer (Cobas Integra 400, Roche Diagnostics, Mannheim Germany) using the commercially available reagent kits supplied by the company. The lipid profile values were classified according to the National Cholesterol Education Programme (ATP-3) report (2002). Plasma aldosterone levels were quantified from the plasma of untreated individuals using ELISA kits (Diagnostics Biochem, Dorchester, Ontario, Canada) and the normal reference range was considered as mentioned by Iverson *et al.* (2007).

Genomic DNA isolation and genetic analysis

The genomic DNA was extracted from the whole blood using the standard phenol–chloroform method (Sambrook

and Russell 2001). The *CYP11B2* C-344T polymorphism was determined by PCR- RFLP. The primers used were 5′-CAG GGC TGA GAG GAG TAA AA-3′ (forward) and 5′-CAG GGG GTA CGT GGA CAT TT-3′ (reverse) as reported by Vasudevan *et al.* (2011) and their protocol was followed with some modifications. The PCR amplification was performed in a 20 μL reaction mixture containing 5 pmol each of forward and reverse primers, 1.9 nM of each dNTP, 1× *Taq* buffer A (10× buffer A (Banglore Genei, Bangalore, India): 100 mM Tris (pH 9.0), 500 mM KCl, 15 mM $MgCl_2$, 0.1% gelatin) 1.25 mM $MgCl_2$, 1.5 U *Taq* DNA polymerase (Bangalore Genei) and 3 μL of template DNA. The thermal cycling conditions included initial denaturation for 10 min at 94°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 min at 72°C. The amplicon was digested with *Hae*III (2.5 units) overnight and subjected to electrophoresis on 3% agarose gel. The digestion of the 152-bp PCR product resulted in the production of 97 and 56 bp bands in the presence of C allele whereas the product remains uncut in presence of T allele.

The A1166C polymorphism of *ATR1* was detected by PCR-RFLP according to Araújo *et al.* (2004) with certain alternations. The primers used were 5′-AAT GCT TGT AGC CAA AGT CAC CT-3′ (forward) and 5′-GGC TTT GCT TTG TCT TGT TG-3′ (reverse). The reaction mixture composition was the same as stated earlier with the only exception that 1.25 mM $MgCl_2$ was not added. The thermal cycling conditions included an initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, elongation at 72°C for 90 s and a final extension at 72°C for 10 min. The amplified PCR product was identified to be 856 bp in size. It was digested with *Dde*I (2.5 units) overnight and separated on 3% agarose gel. The presence of 600 and 256 bp bands represented the homozygous A allele whereas in the presence of the C allele, the 256 bp band was further cut into 146 and 110 bp bands.

The *11βHSD2* G534A polymorphism was determined using the protocol and primers (forward 5′-AGG ACA CGG GGA CTG GAA G-3′ and a reverse 5′-GGG GGC TCC TTT TTG CTC C-3′) published by Mune *et al.* (1995) and Smolenicka *et al.* (1998) and with certain modifications in the protocol. The composition of the reaction mixture was same as that used to detect the *ATR1* A1166C polymorphism. The cycling conditions involved an initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 20 s, annealing at 58°C for 15 s, elongation at 72°C for 30 s and a final extension at 72°C for 7 min. The amplified PCR product was identified as a 293 bp band and the products were digested with *Alu*I (2.5 units) overnight and separated on 2% agarose gel. The amplified PCR product was identified as a 293 bp band The presence of 125, 76, 66 and 26 bp (not visible) bands represents G allele and 151, 76 and 66 bp bands represents the A allele.

Statistical analysis

Unpaired *t*-test / chi-square test / Fisher's tests were used to compare the characteristics of two groups. To compare the aldosterone levels of two groups, Mann–Whitney U test and to compare aldosterone levels according to different genotypes, Kruskal–Wallis test were used since the distribution of the values deviated from the normal. Genotypes and alleles were compared using chi-square test or Fisher's exact test as applicable. Graph Pad ver. 5 (http://www.graphpad.com) was used for the analysis of *CYP11B2* C-344T and *ATR1* A1166C polymorphisms. For the analysis of *11βHSD2* G534T polymorphism, online software available at http://statpages.org/ctab2x2.html was used due to the presence of zero in the contingency table. Binary logistic regression analysis was carried out to identify the independent risk factors using SPSS ver. 17 (www.ibm.com/software/analytics/spss). The risk genotypes and the other factors whose values were in the risk range were coded as 1 and nonrisk values were coded as 2.

Results

Characteristics of patients

A total of 246 hypertensive (males, 159; females, 87) and 274 normotensive (males, 158; females, 116) age and sex matched individuals were included in the study. All the individuals belonged to the general population. The SBP, DBP, BMI, triglyceride levels, frequency of overweight/obesity and alcohol consumption showed significant differences being higher among the patients as compared to controls, while HDL levels were significantly high in control group of individuals. However, the mean BMI values in both the groups were higher than the normal cut-off levels (table 1).

Genderwise analysis of the variables indicate that SBP, DBP and frequency of overweight/obesity were higher in hypertensive cases as compared to normotensive cases in both males and females. But the triglyceride levels and rate of alcohol consumption were high in male patients whereas BMI, creatinine levels and rate of hyperlipidemia were high in female patients.

Genotyping results and aldosterone levels

On analysing the *CYP11B2* C-344T polymorphism, the frequencies of TT, TC and CC genotypes were found to be 51.47, 39.71 and 8.82% among the hypertensives and 72.95, 19.26 and 1.93% among normotensives, respectively, while the frequencies of T and C alleles were 71.32 and 28.68% among the former and 85.51 and 14.49% among the later group of subjects. The frequencies of TT, TC and CC were: hypertensive males: 48.89, 40.00 and 11.11%, normotensive males: 80.51, 16.95 and 2.54%, hypertensive females: 56.52, 39.13 and 4.35% and normotensive females: 62.92, 35.96 and 1.12%. The allele frequencies of T and C were: in male patients: 68.89 and 31.11%, male controls: 88.98 and 11.02%, female patients: 76.09 and 23.91% and female

Table 1. Characteristics of patients and controls.

Variables	Total hypertensives ($n = 246$)	Total normotensives ($n = 274$)	Male hypertensives ($n = 159$)	Male normotensives ($n = 158$)	Female hypertensives ($n = 87$)	Female normotensives ($n = 116$)
Age	49.47 ± 10.38	48.82 ± 11.04	49.20 ± 9.76	47.17 ± 9.25	49.87 ± 11.47	51.30 ± 13.15
SBP (mmHg)	148.4 ± 18.40	116.2 ± 5.40***	149.8 ± 17.11	115.9 ± 5.47 ***	146.5 ± 20.03	116.5 ± 5.33 ***
DBP (mmHg)	93.18 ± 9.94	78.14 ± 4.24***	93.34 ± 10.28	78.19 ± 4.57 ***	92.96 ± 9.50	78.07 ± 3.85 ***
Family history of HTN (%)	42.22	12.5***	46.67	11.11***	33.33	14.29**
BMI (kg/m^2)	24.23 ± 3.99	23.16 ± 1.94**	24.15 ± 4.34	23.49 ± 2.09	24.40 ± 3.18	22.72 ± 1.63 ***
Overweight/obese (%)	63.01	40.98***	63.27	40.00***	62.50	42.31**
TC (mmol/L) (BMI ≥23 kg/m^2)	175.7 ± 31.80	172.1 ± 34.75	4.71 ± 0.80	4.66 ± 1.01	4.34 ± 0.82	4.25 ± 0.72
HDL (mmol/L)	0.99 ± 0.23	1.09 ± 0.34*	1.01 ± 0.18	1.09 ± 0.33	0.96 ± 0.28	1.08 ± 0.35
LDL (mmol/L)	2.67 ± 0.61	2.64 ± 0.68	2.70 ± 0.54	2.77 ± 0.77	2.62 ± 0.71	2.49 ± 0.54
TG (mmol/L)	1.94 ± 0.92	1.60 ± 0.87*	2.17 ± 1.07	1.73 ± 0.88 *	1.65 ± 0.58	1.46 ± 0.85
HDL/LDL	0.3961 ± 0.16	0.4477 ± 0.21	0.3880 ± 0.10	0.4375 ± 0.22	0.4070 ± 0.23	0.4587 ± 0.20
Urea (mmol/L)	7.20 ± 3.18	6.95 ± 2.10	7.28 ± 3.46	7.19 ± 2.39	6.73 ± 2.84	6.70 ± 1.73
Creatinine (μmol/L)	78.72 ± 24.75	77.17 ± 20.33	88.03 ± 28.75	84.86 ± 27.40	66.30 ± 19.45	71.54 ± 11.49*
Hyperlipidemia (%)	76.19	64.52	66.67	68.75	88.89	60.00*
Smoking (%)	20.73	18.33	32.08	34.92	0	0[#]
Chewable tobacco consumption (%)	25.61	21.67	35.85	34.38	6.90	7.14
Alcohol consumption (%)	19.51	10.00**	30.19	18.75*	0	0[#]
Aldosteorne levels (pmol/L)	330.94 ± 103.61	271.41 ± 126.72**	349.80 ± 110.27	254.65 ± 150.82**	309.02± 93.32	290.16 ± 93.96

Continuous variables are expressed in mean ± standard deviation (SD) and frequency data are expressed in %.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; HDL/LDL, high density lipoprotein / low density lipoprotein ratio; mmol/L, millimole/litre
[#]Females were nonsmokers and nonalcoholics. *$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$.

controls: 80.90 and 19.10%. All the genotype distributions were in Hardy–Weinberg equilibrium (HWE).

The genotype patterns of the *CYP11B2* C-344T polymorphism between patients and controls groups were found to be significantly different in the pooled and male populations ($P < 0.0001$ in both cases). From univariate analysis, the polymorphism was found to be associated with hypertension in the entire population in dominant ($P < 0.0001$, OR = 2.542, CI: 1.68–3.84), recessive ($P = 0.0019$, OR = 4.911, CI: 1.63–14.78) and additive ($P = 0.0002$, OR = 6.471, CI: 2.13–19.67) models. In males, significant differences were found between the genotype patterns ($P < 0.0001$) and allele frequency distributions. The associations were observed in dominant ($P < 0.0001$, OR = 4.381, CI: 2.45–7.61), recessive ($P = 0.0123$, OR = 4.792, CI:1.35–16.99) and additive ($P = 0.0008$, OR = 7.197, CI: 2.003–25.86) models. The C allele was also found to be higher in the entire group ($P < 0.0001$, OR = 2.372, CI:1.68–3.36) as well as in male patients ($P < 0.0001$, OR = 3.648, CI: 2.25–5.91) (figure 1a; tables 2 & 3).

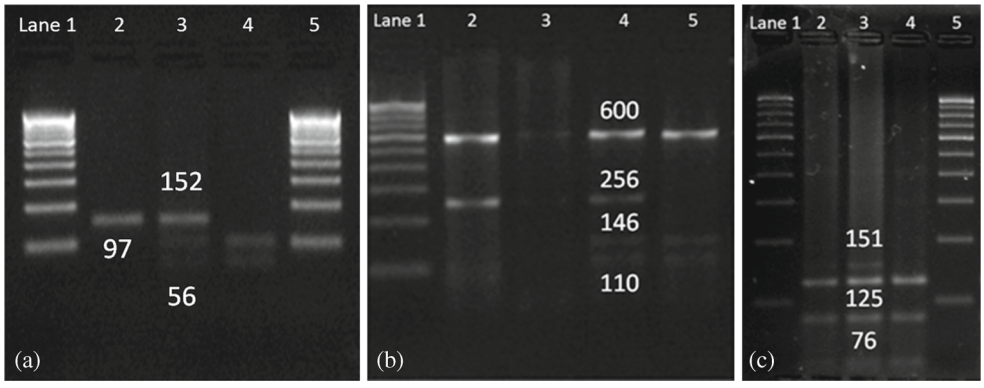

Figure 1. Gel photographs showing banding patterns of different polymorphisms (a) CYP11B2 C-344T, lanes 1 and 5, ladder; lane 2, TT; lane 3, TC; lane 4, CC. (b) ATR1 A1166C: lane 1, ladder; lanes 2 and 3, AA; lane 4, AC; lane 5, CC. (c) 11βHSD2 G534A: lanes 1 and 5, ladder; lanes 2 and 4, GG; lane 3, GA.

Table 2. Genotype and allele frequencies of *CYP11B2* C-344T polymorphism.

	All subjects		Male		Female	
	Patient	Control	Patient	Control	Patient	Control
Genotype						
TT	105 (51.47%)	151 (72.95%)	66 (48.89%)	95 (80.51%)	39 (56.52%)	56 (62.92%)
TC	81 (39.71%)	52 (19.26%)	54 (40.00%)	20 (16.95%)	27 (39.13%)	32 (35.96%)
CC	18 (8.82%)	4 (1.93%)	15 (11.11%)	3 (2.54%)	3 (4.35%)	1 (1.12%)
P value	< 0.0001		< 0.0001		0.3742	
Allele						
T	291 (71.32%)	354 (85.51%)	186 (68.89%)	210 (88.98%)	105 (76.09%)	144 (80.90%)
C	117 (28.68%)	60 (14.49%)	84 (31.11%)	26 (11.02%)	33 (23.91%)	34 (19.10%)
OR (95%CI), *P* value	2.372 (1.68–3.36), < 0.0001		3.648 (2.25–5.91), < 0.0001		1.331 (0.77–2.29), 0.2993	

OR, odds ratio; 95% CI, 95% confidence interval.

Table 3. Association of different genotypes/genetic models of *CYP11B2* C-344T polymorphism with hypertension.

	All subjects		Male		Female	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
TC vs TT	2.242 (1.46–3.44)	0.0002	3.886 (2.13–7.09)	< 0.0001	1.212 (0.63–2.33)	0.5658
CC vs TC	2.889 (0.93–9.02)	0.0915	1.852 (0.48–7.09)	0.5455	3.556 (0.35–36.22)	0.3396
CC vs TT	6.471 (2.13–19.67)	0.0002	7.197 (2.003–25.86)	0.0008	4.308 (0.43–42.98)	0.3085
CC/TC vs TT	2.542 (1.68–3.84)	< 0.0001	4.318 (2.45–7.61)	< 0.0001	1.305 (0.69–2.48)	0.4152
CC vs TC/TT	4.911 (1.63–14.78)	0.0019	4.792 (1.35–16.99)	0.0123	4.000 (0.41–39.35)	0.3189

OR, odds ratio, 95% CI, 95% confidence interval.

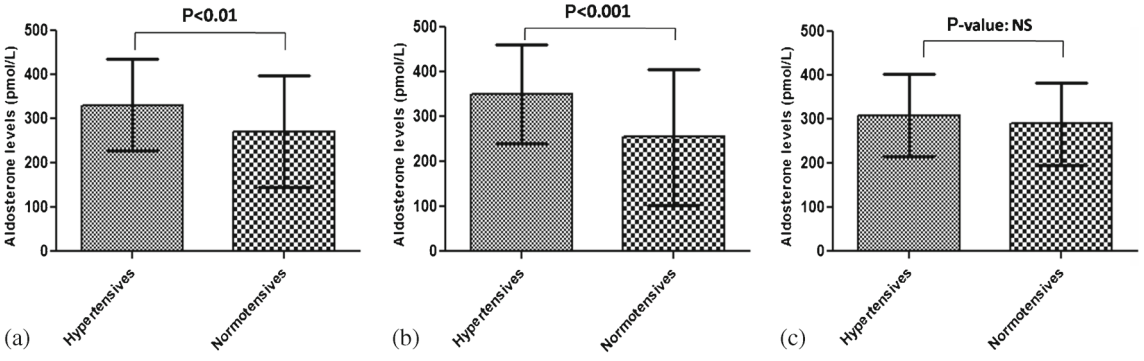

Figure 2. Aldosterone levels in hypertensives (patients) and normotensives (controls) among the (a) total population; (b) males and (c) females.

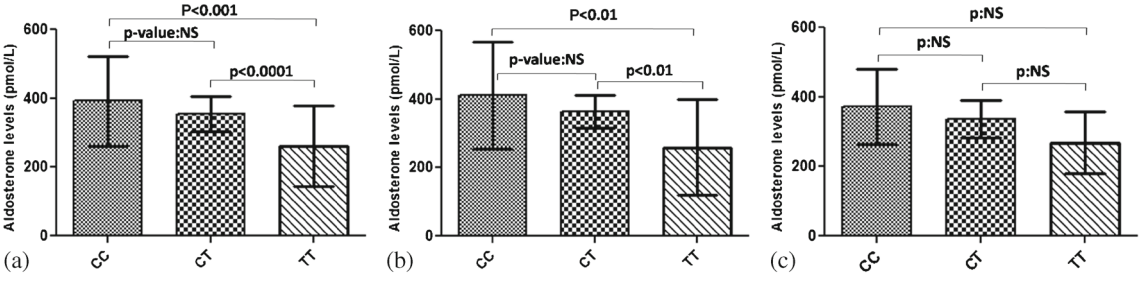

Figure 3. Association of aldosterone levels with different genotypes among the (a) total population (Kruskal–Wallis test *P* value< 0.0001), (b) males (Kruskal–Wallis test *P* value< 0.001) and (c) females (Kruskal–Wallis test *P* value: not significant).

Plasma aldosterone levels were significantly high in the total group of hypertensive patients ($P = 0.0024$) and hypertensive males ($P = 0.0004$) compared to respective controls. The association persisted even after logistic regression analysis ($P = 0.040$ and 0.024, respectively) but in females the levels were not different (figure 2). Though a gradation in the levels of aldosterone was observed according to genotypes based on Kruskal–Wallis analysis in the total group and males (the highest being in CC and lowest in TT genotypes) the aldosterone levels of CC and CT were not significantly different. In females, no differences were observed (figure 3).

In *ATR1* A1166C polymorphism, the genotype frequencies for AA, AC and CC were 83.33, 15.00 and 1.67% in hypertensives and 92.35, 7.65 and 0% in normotensives. In males, the genotype frequencies were 84.62, 12.82 and 2.56% in patient group and 90.91, 9.09 and 0% in control group and in females the respective frequencies were 80.95, 19.05 and 0 and 95.00, 5.00 and 0.0%. The distributions were in HWE except in male patients ($\chi^2 = 7.23$, $P < 0.01$). Since very low frequency of CC genotype was observed in patients and limited to males only, and was completely absent in the control group, CC genotype was pooled with AC genotype for analysis. The AC/CC genotypes were associated with hypertension in the total population ($P = 0.0073$, OR = 2.415, CI: 1.25–4.67) and in females ($P = 0.0225$, OR = 4.471, CI: 1.24–16.12). The C allele was also higher in hypertensives in the pooled ($P = 0.0030$, OR = 2.538, CI: 1.35–4.79) as well as in female population ($P = 0.0277$, OR = 4.105, CI: 1.17–14.43) (figure 1b; table 4).

Analysis of the *11βHSD2* G534A polymorphism revealed that the homozygous mutant genotype (AA) was completely absent and the heterozygous genotype (GA) was present in a very low frequency (4.62%) that to in hypertensives (2.27% in males and 9.52% in females) and the wild-type (GG) frequency was 95.38, 97.73 and 90.48% in total, male and female hypertensives, respectively (table 4). The frequency of GA genotype and A allele was higher in the total group ($P = 0.001$, OR = 0.0, CI: 0.0–0.53) as well as in males ($P = 0.005$, OR = 0.0, CI: 0.0–0.66). The A allele was also higher in hypertensives of both the groups ($P = 0.001$, OR = 0.0, CI: 0.0–0.54 and $P = 0.005$, OR = 0.0, CI: 0.0–0.65, respectively) but no difference was observed in females. The odds ratio was observed to be zero due to the presence of zero in one of the cells in Fisher's test since the GA genotype was absent in controls (figure 1c; table 5).

Table 4. Genotype and allele frequencies of *ATR1* A1166C polymorphism and its association with hypertension.

	All subjects				Male				Female			
	Patient	Control	OR (95%CI)	*P* value	Patient	Control	OR (95%CI)	*P* value	Patient	Control	OR (95%CI)	*P* value
Genotype												
AA	200 (83.33%)	157 (92.35%)			132 (84.62%)	100 (90.91%)			68 (80.95%)	57 (95.00%)		
AC	36 (15.00%)	13 (7.65%)	2.174 (1.12–4.24)	0.0203*	20 (12.82%)	10 (9.09%)	1.515 (0.68–3.38)	0.3076*	16 (19.05%)	3 (5.00%)	4.471 (1.24–16.12)	0.0225*
CC	4 (1.67%)	0 (0%)	2.415 (1.25–4.67)	0.0073**	4 (2.56%)	0 (0%)	1.818 (0.83–3.97)	0.13**	0	0	4.471 (1.24–16.12)	0.0225**
Allele												
A	436 (90.83%)	327 (96.18%)			284 (91.03%)	210 (95.45%)			152 (90.48%)	117 (97.50%)		
C	44 (9.17%)	13 (3.82%)	2.538 (1.35–4.79)	0.0030	28 (8.97%)	10 (4.55%)	2.07 (0.98–4.36)	0.0508	16 (9.52%)	3 (2.50%)	4.105 (1.17–14.43)	0.0277

*P value and odds-ratio calculated for AC vs AA. **P value and odds-ratio calculated for AC/CC vs AA. OR, odds ratio; 95% CI, 95% confidence interval.

Logistic regression analysis results

Logistic regression analysis revealed that in total population, the *CYP11B2* CT/TT ($P = 0.006$, Exp(B): 1.096, 95% CI: 1.027–1.169) and ATR1 AC/CC ($P < 0.001$, Exp(B): 1.128, 95% CI: 1.063–1.197) genotypes and alcohol consumption ($P < 0.001$, Exp(B): 3.901, 95% CI: 2.013–7.560) were independently associated with essential

Table 5. Genotype and allele frequencies of *11βHSD2* G534A polymorphism and its association with hypertension.

	All subjects				Male				Female			
	Patient	Control	OR (95%CI)	*P* value	Patient	Control	OR (95%CI)	*P* value	Patient	Control	OR (95%CI)	*P* value
Genotype												
GG	186 (95.38%)	212 (100%)	0.0 (0.0–0.53)	0.001*	129 (97.73%)	124 (100%)	0.0 (0.0–2.37)	0.248*	57 (90.48%)	88 (100%)	0.0 (0.0–0.66)	0.005*
GA	9 (4.62%)	0			3 (2.27%)	0			6 (9.52%)	0		
AA	0	0			0	0			0	0		
Allele												
G	381 (97.69%)	424 (100%)			261 (98.86%)	248 (100%)			120 (95.24%)	176 (100%)		
A	9 (2.31%)	0	0.0 (0.0–0.54)	0.001	3 (1.14%)	0	0.0 (0.0–2.44)	0.250	6 (4.76%)	0	0.0 (0.0–0.65)	0.005

**P* value and odds-ratio calculated for GA vs GG. OR, odds ratio; 95% CI, 95% confidence interval. OR was observed to be zero due to presence of zero in one of the cells in Fisher's test since GA genotype was absent in controls.

hypertension. In males *CYP11B2* CT/TT genotypes ($P < 0.001$, Exp(B): 1.242, 95% CI: 1.124–1.372) and alcohol consumption ($P < 0.001$, Exp(B): 3.842, 95% CI: 1.888–7.815) and low HDL levels ($P = 0.032$, Exp(B): 3.983, 95% CI: 1.129–14.050) and in females, only the *ATR1* AC/CC genotypes ($P < 0.001$, Exp(B): 1.202, 95% CI: 1.095–1.320) were found to be independent risk factors. High HDL/LDL ratio was however associated with hypertensives in the total group ($P = 0.009$, Exp(B): 0.264, 95% CI: 0.097–0.720) as well as in males ($P = 0.001$, Exp(B): 0.038, 95% CI: 0.006–0.257). Aldosterone levels were also found to be high in hypertensives in both the groups ($P = 0.040$, Exp(B): 1.079, 95% CI: 1.003–1.160 and $P = 0.024$, Exp(B): 1.866., 95% CI: 1.087–3.206, respectively) (table 6).

Discussion

Essential hypertension (EH) is a polygenic disorder. There are most likely several causal genes, which together account for 30 to 50% variation in blood pressure detected among individuals. EH subjects appear to have inherited an aggregate of genes related to hypertension and/or to have been exposed to exogenous factors that predispose them to hypertension. It is widely accepted that knowledge on candidate gene polymorphisms and their association with the pathophysiology of essential hypertension can help take up proper interventional/treatment strategies. Therefore this study was carried out in a subset of Odisha population for the first time to know the association of genetic polymorphisms of *CYP11B2*, *ATR1* and *11βHSD2* genes and their relationship with essential hypertension.

Our study has revealed a strong association between C allele of *CYP11B2* (−344C/T) gene and hypertension (adjusted $P = 0.006$) which is similar to the earlier findings in a Japanese population by Tsukada *et al.* (2002). Gender-wise analysis of the data revealed that males harbouring C allele were at greater risk (adjusted $P = 0.000$) for developing hypertension, while no association could be observed in females. In contrast, Rajan *et al.* (2010) in Tamil Nadu, India, have reported T allele to be linked to blood pressure in the total group and males but not in females; and Xu *et al.* (2004) in China have reported C allele to confer risk in females. Hu *et al.* (2006) from China, however, failed to report any association. The aldosterone levels in our study population were significantly high in patients in the pooled group and males compared to controls even after adjusting for other factors which was not observed in females. Further, the association of genotype-aldosterone levels observed in our study population (highest in CC and lowest in TT) is similar to that observation made by Pojoga *et al.* (1998) in an European population. There have been other studies reporting the reverse trend with highest levels in TT and lowest in CC (Tsukada *et al.* 2002, Japan) and still others reporting no association (Brand *et al.* 1999, Germany). The significance of the role of this polymorphism in the expression of

Table 6. Results of logistic regression analysis.

	All subjects				Male				Female			
			95% CI for exp (B)				95% CI for exp (B)				95% CI for exp (B)	
Factor	*P*	Exp(B)	Lower	Upper	*P*	Exp(B)	Lower	Upper	*P*	Exp(B)	Lower	Upper
CYP11B2 C-344T	0.006	1.096	1.027	1.169	< 0.001	1.242	1.124	1.372	0.406	0.959	0.868	1.059
ATR1 A1166C	< 0.001	1.128	1.063	1.197	0.146	1.063	0.979	1.155	< 0.001	1.202	1.095	1.320
11βHSD2 G534A	0.255	1.073	0.951	1.211	0.851	1.010	0.914	1.115	0.712	1.019	0.921	1.128
BMI	0.971	1.002	0.915	1.096	0.360	1.067	0.929	1.225	0.426	0.949	0.833	1.080
Smoking	0.054	0.525	0.273	1.012	0.081	0.544	0.274	1.079	−*			
Smokeless tobacco	0.656	0.897	0.557	1.445	0.905	0.968	0.564	1.662	0.233	1.332	0.831	2.133
Alcohol	< 0.001	3.901	2.013	7.560	< 0.001	3.842	1.888	7.815	−*			
TC	0.414	1.506	0.564	4.019	0.254	2.506	0.516	12.166	0.354	0.419	0.067	2.637
HDL	0.559	1.243	0.600	2.575	0.032	3.983	1.129	14.050	0.180	0.421	0.119	1.492
LDL	0.607	0.818	0.381	1.758	0.342	0.437	0.079	2.408	0.413	1.551	0.543	4.432
Triglycerides	0.091	2.132	0.886	5.134	0.125	4.850	0.647	36.367	0.065	3.715	0.922	14.973
HDL/LDL ratio	0.009	0.264	0.097	0.720	0.001	0.038	0.006	0.257	0.798	0.823	0.186	3.639
Aldosterone level	0.040	1.079	1.003	1.160	0.024	1.866	1.087	3.206	0.189	1.072	0.966	1.189
Constant	0.246	0.566			0.606	0.729			0.333	2.079		

*Females were nonsmokers or nonalcoholics; P significance, or other abbreviations used are similar to table 1. Exp(B), exponentiation of the B coefficient, which is an odds ratio.

aldosterone synthase is controversial. The –344C/T polymorphism at the SF-1 site is thought to alter the sensitivity of aldosterone synthase to angiotensin II (Davies *et al.* 1999). Although the −344C allele binds the SF-1 five times more than the T allele, but the polymorphism in itself may have no impact on the transcriptional regulation of aldosterone synthase. It is possible that the increased transcription factor (SF-1) availability at other functional sites (e.g. positions −71, −64) might result in altered expression of aldosterone synthase. Other mechanisms, including linkage of the polymorphism with a quantitative trait locus elsewhere in the regulatory region need to be explored or it might only become functional through epigenetic interaction with other genes (Staessen *et al.* 2007).

The *ATR1* (A1166C) C allele was associated with hypertension in the total as well as female populations (adjusted *P* value<0.001 in both cases), whereas no association was found in male populations. Similar to our observation, Bonnardeaux *et al.* (1994) have reported a positive association between the C allele and essential hypertension in Caucasians in France in contrast to the findings of Castellano *et al.* (1996) who have observed A allele to be the risk factor for essential hypertension in Italy. Gender specific available literature indicates a significant association of the A allele with hypertension in Tibetan males but not in females (Liu *et al.* 2003) and C allele with hypertension in Siberian males (Stanković *et al.* 2003) whereas in Iran, A allele was higher in patients with metabolic syndrome (Alavi-Shahri *et al.* 2010). Tsai *et al.* (2003), however, found no association in Taiwan and similar to our study the CC genotype was absent from controls. The frequency of C allele was low in our population under study (0.01) similar to north Indian (0.09) (Kaur *et al.* 2012) and Tibetan populations (0.04) (Liu *et al.* 2003). However higher frequencies have been reported in south India (0.27) (Pullareddy *et al.* 2009) and Turkey (0.18) (Agachan *et al.* 2003).

The mechanism responsible for the association of hypertensive status with the A1166C polymorphism generally remains unknown. Since the polymorphism is in a noncoding region of the gene, the amino acid sequence of the receptor remains unaltered. It has been hypothesized that it might affect mRNA stability and transcription, or alternatively be in linkage disequilibrium with other polymorphisms (Jira *et al.* 2010). Recently, it has been shown to interrupt the ability of microRNA-155 to attenuate translation, thereby leading to augmented ATR1 expression which can lead to development of cardiovascular disease (Martin *et al.* 2007).

The *11βHSD2* polymorphism-derived allele frequency was very low, completely absent in controls and no homozygous mutants were found. After adjustment of all factors, no link of this polymorphism with hypertension could be detected. Similar to our study, very low frequency of A allele and absence of the AA genotype have been reported in Italy and Switzerland and no association could be detected (Smolenicka *et al.* 1998; Mariniello *et al.* 2005). But frequency of GG genotype was found to be higher in patients with primary hypertension than in normotensive control subjects in Sweden (Melander *et al.* 2000).

The differences observed in genotypic associations with pathophysiological conditions among different populations may be due to race, age, gender, sampling methods, genetic epitasis, linkage with other polymorphism(s) and environmental factors. The gender-specific association may be due to linkage of the polymorphism to some other variant(s) in some autosomal or sex chromosome or due to the effect of gonadial hormones or some environmental factors. Since essential hypertension is also influenced by environmental factors, some of these factors were also examined. In the total

population, alcohol consumption was identified as risk factors for essential hypertension. In males, alcohol consumption and low HDL levels were observed to contribute to the risk but in females no environmental factors could be identified. However, high HDL/LDL ratio which is generally a protective factor was associated with hypertensives which may be due to high levels of LDL in male controls.

The main limitation of our study is the sample size. A larger sample would be required to reach to a more accurate conclusion. Besides, few other biochemical and protein expression studies like cortisol, cortisone estimation and enzyme activity analysis can also add to the knowledge. In conclusion, it was found that in males *CYP11B2* C-344T polymorphism, alcohol consumption and low HDL levels and in females, *ATR1* A1166C polymorphism were associated with essential hypertension in our study population.

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