CLINICAL STUDY

Association of *HSD3B1* and *HSD3B2* gene polymorphisms with essential hypertension, aldosterone level, and left ventricular structure

Masanori Shimodaira^{1,2}, Tomohiro Nakayama^{1,3,4}, Naoyuki Sato³, Noriko Aoi^{3,4}, Mikano Sato^{3,4}, Yoichi Izumi⁴, Masayoshi Soma^{4,5} and Koichi Matsumoto⁴

¹Division of Laboratory Medicine, Department of Pathology of Microbiology, Nihon University School of Medicine, 30-1 Ooyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan, ²Division of Hematology, Endocrinology and Metabolism, Tokyo Metropolitan Hiroo Hospital, 2-34-10 Ebisu, Shibuya-ku, Tokyo 150-0013, Japan, ³Division of Molecular Diagnostics, Department of Advanced Medical Science, Divisions of ⁴Nephrology and Endocrinology and ⁵General Medicine, Department of Medicine, Nihon University School of Medicine, 30-1 Ooyaguchi-kamimachi, Itabashi-ku, Tokyo 173-8610, Japan

(Correspondence should be addressed to T Nakayama; Email: tnakayam@med.nihon-u.ac.jp)

Abstract

Background: *HSD3B1* and *HSD3B2* are crucial enzymes for the synthesis of hormonal steroids, including aldosterone. Therefore, *HSD3B* gene variations could possibly influence blood pressure (BP) by affecting the aldosterone level.

Methods: We performed a haplotype- and diplotype-based case-control study to investigate the association between the *HSD3B* gene variations and essential hypertension (EH), aldosterone level, and left ventricular hypertrophy (LVH). A total of 275 EH patients and 286 controls were genotyped for four SNPs of the *HSD3B1* gene (rs3765945, rs3088283, rs6203, and rs1047303) and for two SNPs of the *HSD3B2* gene (rs2854964 and rs1819698). Aldosterone and LVH were investigated in 240 and 110 subjects respectively.

Results: Significant differences were noted for the total and the male subject groups for the recessive model (CC versus TC + TT) of rs6203 between the controls and EH patients ($P=0.030$ and $P=0.008$ respectively). The frequency of the T-C haplotype established by rs3088283-rs1047303 was significantly higher for EH patients compared with the controls ($P=0.014$). Even though the polymorphism of *HSB3B1* was not associated with LVH, the diplotype established by rs3088283-rs1047303 in the total subject group, along with the systolic BP, diastolic BP, and aldosterone level were significantly higher for those subjects who had the T-C haplotype versus those who did not ($P=0.025$, $P=0.014$, and $P=0.006$ respectively).

Conclusion: rs6203 and rs1047303 in the *HSD3B1* gene are useful genetic markers for EH, while polymorphisms of *HSD3B1* are associated with the BP and aldosterone level.

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Introduction

It is likely that essential hypertension (EH) is a polygenic disorder that results from the inheritance of a number of susceptibility genes. The causal genes identified may be responsible for 30–50% of the observed inter-individual variations in blood pressure (BP) (1). Subphenotypic stratification and measurement of biochemical and physiological intermediate phenotypes provide an important way to dissect out these genetic factors.

Aldosterone is a potent mineralocorticoid that promotes sodium retention and leads to elevation of the arterial pressure. Additionally, aldosterone may also play a role in cardiac hypertrophy that is independent of its effect on BP. Therefore, genetic variations in the

regulation of aldosterone synthesis might influence the structure and function of the left ventricle.

The 3 β -hydroxysteroid dehydrogenases (3 β -HSD)/ $\Delta^{4,5}$ -isomerase is the enzyme responsible for catalyzing the 3 β -HSD dehydrogenation and $\Delta^{4,5}$ -isomerization of the Δ^5 -steroid precursors into their respective Δ^4 -ketosteroids (2). This activity is crucial for the synthesis of hormonal steroids, which includes aldosterone, cortisol, and testosterone. The two 3 β -HSD isoenzymes are chronologically designated as type 1 and type 2. These isoenzymes are 93.5% homologous and encoded by two different genes that are located on chromosome 1p13.1 (3). The type 1 gene (*HSD3B1*) is almost exclusively expressed as 3 β -HSD in the placenta and peripheral tissues, including in the mammary gland, the prostate, and the skin. In contrast, the type 2

gene (*HSD3B2*) is predominantly expressed as 3 β -HSD in the adrenal gland, ovary, and testis (4).

A recent study in mice shows that type VI 3 β -HSD (*Hsd3b6*), which is functionally similar to human *HSD3B1*, is associated with hypertension (HT) (5). In humans, a genetic variation in *HSD3B1* can lead to an elevation in plasma aldosterone with a resultant increase in intravascular volume and HT (6). In this study, it was reported that rs6203, which is the T→C silent substitution at codon 338 in exon 4 of *HSD3B1*, was associated with an elevated BP in a population of Swedish men. These previous studies indicate that the *HSD3B1* and *HSD3B2* genes may modulate BP and cardiac hypertrophy via changes in circulating aldosterone. To clarify this, we attempted to determine if the common SNPs and haplotypes/diplotypes of these genes were associated with BP and aldosterone levels. In addition, we also examined the differences in the echocardiographic left ventricular hypertrophy (LVH) between EH patients with normal BP and those who had never been treated for EH.

Materials and methods

Subjects

Subjects diagnosed with EH were recruited at Nihon University Itabashi Hospital and at other neighboring hospitals in Tokyo from 1993 to 2003. We enrolled 275 EH patients in the present study, with a male/female (m/f) ratio of 1.89. All the subjects had been previously screened for EH and had undergone physical and laboratory examinations. To be diagnosed with EH, the subjects had to have a family history of HT and a seated systolic BP (SBP) >160 mmHg or a diastolic BP (DBP) >100 mmHg on three occasions within 2 months after their first BP reading. A family history of HT was defined as prior diagnosis of HT in grandparents, uncles, aunts, parents, or siblings. None of the EH patients were receiving antihypertensive medications. Any patients diagnosed with secondary HT, including primary aldosteronism, were excluded from the study. A total of 286 normotensive (NT) age-matched healthy individuals (m/f ratio=1.80) were recruited at Nihon University Itabashi Hospital and at other neighboring hospitals from 1993 to 2003 and were enrolled as control subjects. None of the control subjects had any family history of HT, and all the controls had an SBP <130 mmHg and a DBP <85 mmHg. All EH subjects and controls were Japanese. Informed consent was obtained from each subject in accordance with the protocol approved by the Ethics Committee of Nihon University (7). This investigation was performed in line with the guidelines of the Declaration of Helsinki.

SNP selection and genotyping

A total of six SNPs were selected from the public databases available at the NCBI dbSNP and at the International HapMap Project websites (<http://www.ncbi.nlm.nih.gov> and <http://www.hapmap.org> respectively). The structures of the *HSD3B* genes are shown in Fig. 1. Since the linkage disequilibrium (LD) analysis, which was based on the JPT HapMap (release 19/phaseII October 2005, on NCBI B34 assembly, dbSNP b124), found that the SNPs tagged for each locus were on the same block ($r^2 > 0.8$), and this made it possible to perform a complete linkage block analysis.

Standard methods were used to extract DNA, while the genotyping was performed using a previously described Taq amplification method of the TaqMan SNP Genotyping Assay (Applied Biosystems Inc., Norwalk, CT, USA) (8). In the present study, we genotyped three SNPs that spanned the *HSD3B1* gene and which included, rs3765945 in the intron region (also known as T3216C), rs3088283 in the third exon (also known as The 54 Ile), and rs6203 and rs1047303 in the fourth exon (also known as Leu 338 Leu and as Thr 367 Asn respectively). The two SNPs genotyped in the *HSD3B2* gene included rs2854964 (A1167T) in the second intron and rs1819698 (G7747A) in the UTR-3. Genotyping was carried out blinded to the phenotypic information with controls of the known genotype included in each genotyping run. There was a 90% success rate for the genotyping with higher and minor allele frequencies all >5%. The estimated genotyping error rate was <1%.

Haplotype and diplotype construction

Based on the genotype data for the genetic variations, we performed a LD analysis along with haplotype-based case-control analysis and diplotype construction. These analyses used the expectation maximization

Figure 1 Top, genomic structure of *HSD3B1* and *HSD3B2*. The exons and the introns are indicated by lines and boxes respectively. The sizes of the genes are indicated by the scale at the top of the figure. Bottom, r^2 and pairwise LD (D') estimates are provided in the upper and lower triangles of each box respectively.

Table 1 Characteristics of the study participants. Continuous variables are expressed as means $\pm$ s.d. Categorical variables are expressed as percentages. The *P* value for the continuous variables were calculated using the Mann–Whitney *U* test. The *P* value for the categorical variables were calculated using Fisher's exact test.

	Total			Males			Females		
	NT (n=286)	EH (n=275)	<i>P</i> value	NT (n=184)	EH (n=180)	<i>P</i> value	NT (n=102)	EH (n=95)	<i>P</i> value
Age (years)	51.84 $\pm$ 9.65	51.01 $\pm$ 6.13	0.226	51.73 $\pm$ 6.57	50.94 $\pm$ 6.59	0.253	52.04 $\pm$ 13.58	51.21 $\pm$ 5.21	0.578
BMI (kg/m ²)	22.78 $\pm$ 3.23	27.02 $\pm$ 5.89	<0.0001	22.94 $\pm$ 3.18	24.64 $\pm$ 3.31	<0.0001	22.49 $\pm$ 3.33	24.41 $\pm$ 4.01	0.000
SBP (mmHg)	112.97 $\pm$ 11.70	173.27 $\pm$ 20.46	<0.0001	113.34 $\pm$ 10.65	171.10 $\pm$ 19.09	<0.0001	112.33 $\pm$ 13.41	177.39 $\pm$ 22.28	<0.0001
DBP (mmHg)	69.67 $\pm$ 8.66	106.44 $\pm$ 13.83	<0.0001	70.46 $\pm$ 8.24	107.06 $\pm$ 13.90	<0.0001	68.25 $\pm$ 9.23	105.44 $\pm$ 13.75	<0.0001
Pulse (beats/min)	73.56 $\pm$ 13.05	77.59 $\pm$ 15.01	0.003	72.51 $\pm$ 13.33	77.38 $\pm$ 15.46	0.004	75.88 $\pm$ 12.29	77.99 $\pm$ 14.04	0.336
Creatinine (mg/dl)	0.83 $\pm$ 0.22	0.85 $\pm$ 0.25	0.413	0.90 $\pm$ 0.20	0.94 $\pm$ 0.24	0.162	0.71 $\pm$ 0.18	0.68 $\pm$ 0.18	0.293
Tot. chol. (mg/dl)	198.67 $\pm$ 45.45	210.59 $\pm$ 41.41	0.002	193.63 $\pm$ 44.90	205.32 $\pm$ 42.45	0.014	207.81 $\pm$ 45.27	220.31 $\pm$ 37.54	0.041
HDL chol. (mg/dl)	55.67 $\pm$ 16.88	56.20 $\pm$ 17.80	0.737	53.80 $\pm$ 15.48	52.48 $\pm$ 16.92	0.473	59.07 $\pm$ 18.78	63.03 $\pm$ 17.37	0.153
Uric acid (mg/dl)	5.37 $\pm$ 1.48	5.65 $\pm$ 1.60	0.041	5.82 $\pm$ 1.42	6.20 $\pm$ 1.47	0.016	4.55 $\pm$ 1.20	4.60 $\pm$ 1.28	0.777
PAC (pg/ml)	106.34 $\pm$ 55.44	117.82 $\pm$ 58.87	0.222	108.24 $\pm$ 45.79	115.58 $\pm$ 59.31	0.590	104.87 $\pm$ 62.75	121.91 $\pm$ 58.28	0.222
PRA (pg/ml per h)	3.24 $\pm$ 8.01	3.20 $\pm$ 11.09	0.979	4.60 $\pm$ 10.86	3.28 $\pm$ 11.04	0.612	2.19 $\pm$ 4.75	3.05 $\pm$ 11.26	0.702
ARR	126.81 $\pm$ 126.73	172.23 $\pm$ 267.69	0.254	116.94 $\pm$ 159.29	155.14 $\pm$ 262.63	0.520	134.49 $\pm$ 96.84	203.54 $\pm$ 275.89	0.209
LVDD (mm)	47.56 $\pm$ 6.01	50.57 $\pm$ 6.50	0.183	48.56 $\pm$ 6.01	50.57 $\pm$ 6.50	0.182	47.12 $\pm$ 3.31	50.09 $\pm$ 5.73	0.183
Hyperlipidemia (%)	26.6	40.8	<0.0001	23.4	38.9	0.001	32.4	49.5	0.015
Diabetes (%)	7.3	14.9	0.004	10.0	15.6	0.098	2.9	13.7	0.006
Drinking (%)	38.8	62.2	<0.0001	48.4	76.1	<0.0001	21.6	35.8	0.030
Smoking (%)	28.0	51.3	<0.0001	35.9	62.2	<0.0001	13.7	30.5	0.004

NT, normotension; EH, essential hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Tot. chol., total cholesterol; HDL chol., high density lipoprotein cholesterol; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ARR, PAC to PRA ratio; LVDD, left ventricular diastolic dimensions.

algorithm (9) and the SNPalyze version 3.2 software (Dynacom Co., Ltd, Yokohama, Japan). A total of six SNPs were used for the pairwise LD analysis, with $|D'|$ values >0.5 used to assign SNP locations to a single haplotype block. SNPs with a r^2 value <0.5 were selected as tagged. In the haplotype-based case-control analysis, haplotypes with a frequency <0.02 were excluded. A diplotype was defined as a pair of haplotypes in which one haplotype comes from the mother, while the other comes from the father. Diplotypes for each subject were also constructed by applying SNPalyze version 3.2 (Dynacom Co., Ltd) (10).

Biochemical analysis

Standard methods employed by the Clinical Laboratory, Department of Nihon University Hospital were used to measure the total cholesterol, low-density lipoprotein cholesterol, aldosterone level, and renin activity in the plasma, and the creatinine and uric acid concentrations in the serum. Samples used to determine the aldosterone levels and renin activities were obtained from the subjects after they had been in a sitting position for a minimum of 30 min.

Echocardiographic examination

Echocardiography was performed in 110 individuals, (87 of the 287 EH patients and 23 of the 253 age-matched control subjects), using a standardized procedure to measure the wall thickness in 16 left ventricular wall segments. An experienced echocardiographer, who was blinded to the subject's status including genotype, examined each individual in a supine, left lateral position in accordance with the recommendations of the American Society of Echocardiography (11). Left ventricular diastolic dimensions (LVDD) were measured online from the standard 2D-guided M-mode registrations that were determined according to the leading edge convention. The end diastolic phase (R-wave peak of the ECG trace) was considered to define the interventricular septal thickness (IVST) and posterior wall thickness (PWTd). Left ventricular mass (LVM) was calculated using the American Society of Echocardiography's recommended formula for estimation of LVM from 2D-LV linear dimensions:

$$\text{LVM} = 0.8 \times (1.04((\text{LVDD} + \text{PWTd} + \text{IVST})^3 - (\text{LVDD})^3)) + 0.6 \text{ g}$$

To calculate the LVM index (LVMI), the LVM was divided by the body surface area.

Statistical analysis

Based on our initial estimated sample size, the number of patients enrolled was considered to be sufficient for a gene polymorphism study. In our analyses, we obtained

Table 2 Genotype and allele frequencies in normotensives and patients with EH.

Variants	Function	Total				Males				Females			
		NH (n=286)	EH (n=275)	P value		NH (n=184)	EH (n=180)	P value		NH (n=102)	EH (n=95)	P value	
rs3765945	Intron	Genotype											
		TT	81.8	82.5		81.0	79.4			83.3	88.4		
		TC	18.2	17.1		19.0	20.0			16.7	11.6		
		CC	0.0	0.4	0.565	0.0	0.6			0.0	0.0		N.C.
		Dominant model											
		TT	81.8	82.5		81.0	79.4			83.3	88.4		
		TC+CC	18.2	17.5	0.822	19.0	20.6			16.7	11.6		0.307
		Recessive model											
		CC	0.0	0.4		0.0	0.6			0.0	0.0		
		TC+TT	100.0	99.6	0.307	100.0	99.4			100.0	100.0		N.C.
rs3088283	Missense mutation (The 54 Ile)	Allele											
		T	90.9	91.1		90.5	89.4			91.7	94.2		
		C	9.1	8.9	0.915	9.5	10.6			8.3	5.8		0.326
		Genotype											
		TT	94.1	97.1		92.4	96.7			97.1	97.9		
		TC	5.9	2.9		7.6	3.3			2.9	2.1		
		CC	0.0	0.0	N.C.	0.0	0.0			0.0	0.0		N.C.
		Dominant model											
		TT	94.1	97.1		92.4	96.7			97.1	97.9		
		TC+CC	5.9	2.9	0.082	7.6	3.3			2.9	2.1		0.709
rs6203	Silent mutation (Leu 338 Leu)	Recessive model											
		CC	0.0	0.0		0.0	0.0			0.0	0.0		
		TC+TT	100.0	100.0	N.C.	100.0	100.0			100.0	100.0		N.C.
		Allele											
		T	97.0	98.5		96.2	98.3			98.5	98.9		
		C	3.0	1.5	0.085	3.8	1.7			1.5	1.1		0.711
		Genotype											
		TT	45.1	44.4		46.2	43.9			43.1	45.3		
		TC	46.5	41.5		47.8	41.7			44.1	41.1		
		CC	8.4	14.2	0.082	6.0	14.4			12.7	13.7		0.909
rs1047303	Missense mutation (Thr 367 Asn)	Dominant model											
		TT	45.1	44.4		46.2	43.9			43.1	45.3		
		TC+CC	54.9	55.6	0.860	53.8	56.1			56.9	54.7		0.846
		Recessive model											
		CC	8.4	14.2		6.0	14.4			12.7	13.7		
		TC+TT	91.6	85.8	0.030	94.0	85.6			87.3	86.3		0.764
		Allele											
		T	68.4	65.1		70.1	64.7			65.2	65.8		
		C	31.6	34.9	0.246	29.9	35.3			34.8	34.2		0.902
		Genotype											
rs1047303	Missense mutation (Thr 367 Asn)	AA	90.9	85.5		91.3	86.7			90.2	83.2		
		AC	9.1	12.7		8.7	11.7			9.8	14.7		
		CC	0.0	1.8	0.025	0.0	1.7			0.0	2.1		0.221
		Dominant model											
		AA	90.9	85.5		91.3	86.7			90.2	83.2		
		AC+CC	9.1	14.5	0.022	8.7	13.3			9.8	16.8		0.141
		Recessive model											
		CC	0.0	1.8		0.0	1.7			0.0	2.1		
		TC+TT	100.0	100.0	N.C.	100.0	100.0			100.0	100.0		N.C.
		Allele											

Table 2 Continued

Variants	Function	Total				Males				Females			
		NH (n=286)	EH (n=275)	P value		NH (n=184)	EH (n=180)	P value		NH (n=102)	EH (n=95)	P value	
rs2854964	Intron	Recessive model											
		CC	1.8	0.045		0.0	1.7			0.0	2.1		
		AC+AA	98.2			100.0	98.3	0.157		100.0	97.9	0.145	
		Allele											
		A	95.5			95.7	92.5			95.1	90.5		
		C	4.5	0.938		4.3	7.5	0.071		4.9	9.5	0.776	
		Genotype											
		AA	56.0			55.4	56.7			66.7	54.7		
		AT	37.5			39.7	36.1			29.4	40.0		
		TT	6.5	0.532		4.9	7.2	0.564		3.9	5.3	0.230	
rs1819698	UTR-3	Dominant model											
		AA	56.0			55.4	56.7			66.7	54.7		
		AT+TT	44.0	0.561		44.6	43.3	0.813		33.3	45.3	0.086	
		Recessive model											
		TT	6.5			4.9	7.2			3.9	5.3		
		AT+AA	93.5	0.278		95.1	92.8	0.351		96.1	94.7	0.652	
		Allele											
		A	77.0			75.3	74.7			81.4	74.7		
		T	23.0	0.377		24.7	25.3	0.864		18.6	25.3	0.111	
		Genotype											
rs1819698	UTR-3	GG	37.5			39.1	37.2			30.4	37.9		
		GA	48.0			44.6	47.8			50.0	48.4		
		AA	14.5	0.627		16.3	15.0	0.823		19.6	13.7	0.393	
		Dominant model											
		GG	37.5			39.1	37.2			30.4	37.9		
		GA+AA	62.5	0.724		60.9	62.8	0.708		69.6	62.1	0.267	
		Recessive model											
		AA	14.5			16.3	15.0			19.6	13.7		
		GA+GG	85.5	0.343		83.7	85.0	0.732		80.4	86.3	0.266	
		Allele											
rs1819698	UTR-3	G	61.5			61.4	61.1			55.4	62.1		
		A	38.5	0.454		38.6	38.9	0.933		44.6	37.9	0.176	

Allele frequencies are given as percentages. NT, normotension; EH, essential hypertension. N.C. indicates that the calculation of the χ^2 from the contingency table could not be performed due to inclusion of a sample that did not contain any DNA. *Means remained significant *P* value after Bonferroni's correction ($P < 0.0083$).

90% power during the detection of the disequilibrium at the 5% level of significance. Results of a previously published study also confirmed that our sample sizes were appropriate for this type of case-control study (12).

All continuous variables were expressed as the mean $\pm$ s.d. Differences in continuous variables between the EH patients and control subjects were analyzed using the Mann-Whitney *U* test. Differences in categorical variables were analyzed using Fisher's exact test. Differences in the distributions of the genotypes and alleles between the EH patients and control subjects were analyzed using Fisher's exact test. The frequency distribution of the haplotypes was calculated by performing a permutation test using the bootstrap method. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors. Statistical significance was established at $P < 0.05$. All *P* values were adjusted for the number of tests performed by Bonferroni's correction. Statistical analyses were performed using SPSS software for Windows, version 12 (SPSS Inc., Chicago, IL, USA).

Results

In the present study, 275 EH patients and 286 age-matched controls were genotyped for four SNPs that are the genetic markers for the *HSD3B1* gene (rs3765945, rs3088283, rs6203, and rs1047303) and for the two SNPs that are the markers for the *HSD3B2* gene (rs2854964 and rs1819698). Table 1 presents the clinical characteristics of the study participants, while the distributions of the genotypes and alleles of the six SNPs are listed in Table 2. The six SNPs examined in our study population were consistent with the Hardy-Weinberg equilibrium ($P > 0.05$), and the allele

frequency for each of these SNPs was in agreement with the previously reported HapMap data. In males, there was a significant difference for the rs6203 genotype distribution between the EH patients and the control subjects ($P = 0.026$). For the total and male subject groups, there were also significant differences noted for the distribution of the recessive model of rs6203 (CC versus TC+TT) between the EH patients and the control subjects ($P = 0.030$ and 0.008 respectively). In male subjects, the association between the recessive model of rs6203 and EH remained significant at $P < 0.05$ after Bonferroni's correction on the number of tests performed. For rs1047303 in the total subject group, the genotype distribution, the distribution of the dominant model (AA versus AC+CC), and the recessive model (CC versus AC+AA) differed significantly between the EH patients and the controls ($P = 0.025$, $P = 0.022$, and $P = 0.045$ respectively). Dominance and recessiveness of the model were defined by the frequency found for the total control individuals. While rs6203 and rs1047303 are located in the fourth exon of *HSD3B1*, the SNPs located within the *HSD3B2* gene were not associated with EH.

To investigate the effect of the gene polymorphism on BP, we performed a logistic regression analysis that examined age, body mass index (BMI), total cholesterol, presence or absence of diabetes mellitus, and smoking. For the total and the male subject groups, the CC genotype of rs6203 differed significantly between the EH patients and the control subjects ($P = 0.041$ and $P = 0.031$ respectively). There was also a significant difference for the AC+CC genotype of rs1047303 between the EH patients and control subjects of the total group ($P = 0.027$, Table 3).

LD patterns for the *HSD3B1* and *HSD3B2* genes are shown at the bottom of Fig. 1. As all the r^2 values were below 0.5, all the six SNPs were considered to be located in a single haplotype block and, thus, were

Table 3 Odds ratio for hypertension risk factors and genotypes.

Risk factor	Total			Male			Female		
	Odds ratios	95% CI	<i>P</i> value	Odds ratios	95% CI	<i>P</i> value	Odds ratios	95% CI	<i>P</i> value
Odds ratios and 95% CI for hypertension risk factors and the CC genotypes of rs6203									
CC genotype	1.660	0.910–3.024	0.041	2.330	1.037–5.238	0.031	0.979	0.381–2.512	0.964
Age	0.987	0.964–1.011	0.282	0.985	0.950–1.021	0.420	0.989	0.959–1.020	0.497
BMI	1.135	1.073–1.200	<0.001	1.131	1.051–1.216	0.001	1.142	1.039–1.256	0.006
Total cholesterol	1.006	1.002–1.011	0.008	1.004	0.997–1.011	0.274	1.002	0.993–1.011	0.707
Smoking	1.368	1.251–1.593	0.031	3.176	2.030–5.220	<0.001	3.759	1.730–8.120	0.013
Diabetes mellitus	1.659	0.910–3.024	0.098	0.776	0.406–1.484	0.443	0.265	0.069–1.022	0.540
Odds ratios and 95% CI for hypertension risk factors and the AC+CC genotypes of rs1047303									
AC+CC genotype	1.495	0.265–1.922	0.027	0.797	0.382–1.663	0.545	1.947	0.705–5.376	0.198
Age	0.987	0.964–1.010	0.260	0.983	0.949–1.019	0.352	0.991	0.961–1.023	0.582
BMI	1.132	1.070–1.196	<0.001	1.126	1.048–1.210	0.001	1.137	1.033–1.252	0.009
Total cholesterol	1.006	1.002–1.011	0.008	1.004	0.997–1.011	0.286	1.005	0.997–1.013	0.232
Smoking	1.027	1.253–1.544	<0.001	3.193	1.994–5.103	<0.001	2.732	0.701–4.203	0.013
Diabetes mellitus	0.687	0.381–1.237	0.211	0.797	0.382–1.663	0.545	0.270	0.070–1.042	0.570

CI, confidence intervals.

Table 4 The haplotypes with the lowest overall *P* values in each of the groups.

			Frequency of the haplotype			Adjusted <i>P</i> value	Odds ratio for EH (95% CI)	<i>P</i> value
		Overall <i>P</i> value	NT	EH	<i>P</i> value			
Haplotype established by rs3088283-rs1047303 (<i>Haplotype analysis in total subjects</i>)								
rs3088283	rs1047303	0.011						
T	A		0.925	0.918	0.888			
C	A		0.030	0.022	0.112			
T	C		0.045	0.063	0.014	0.056	1.702 (1.008–2.875)	0.047
Haplotype established by rs3088283-rs6203-rs1819698 (<i>Haplotype analysis in male subjects</i>)								
rs3088283	rs6203	rs1819698	0.016					
T	T	G	0.504	0.426	0.057			
T	C	G	0.109	0.192	0.002	0.016	1.661 (0.989–2.788)	0.055
T	T	A	0.192	0.220	0.358			
T	C	A	0.165	0.162	0.876			
C	C	A	0.030	0.000	0.033	0.264	N.C.	N.C.
Haplotype established by rs1047303-rs2854964 (<i>Haplotype analysis in female subjects</i>)								
rs1047303	rs2854964	<0.001						
A	A		0.808	0.677	0.003	0.012	0.513 (0.145–1.812)	0.003
C	A		0.000	0.081	0.001	0.004	N.C.	N.C.
A	T		0.157	0.242	0.039	0.156	1.678 (0.926–3.041)	0.088
C	T		0.035	0.000	0.284			

NT, normotension; EH, essential hypertension; CI, confidence intervals. Haplotype with frequency >0.02 were estimated by using SNPalyze software. The *P* value of haplotype frequency was calculated by permutation test using bootstrap method. Adjusted *P* values were adjusted for the number of tests by Bonferroni's correction. Odds ratios were estimated for haplotypes that occurred at significantly different frequencies between EH and controls with control haplotypes as the reference. N.C. indicates that the calculation could not be performed due to the absence of haplotype.

suitable for use in a haplotype-based case-control study. In a haplotype-based case-control analysis, haplotypes are created via the use of different SNP combinations. Combinations based on the six SNPs used in this study resulted in a total of 51 haplotype patterns. The haplotypes with the lowest overall *P* value in each of the groups are shown in Table 4. For the total subject group, the frequency of the T-C haplotype established by rs3088283-rs1047303 was significantly higher for the EH patients compared with the control subjects (*P*=0.014). The frequency of the T-C-G haplotype established by rs3088283-rs6203-rs1819698 in the male subjects, and the frequency of the C-A haplotype established by rs1047303-rs2854964 in the female subjects were also significantly higher for the EH patients compared with the control subjects (*P*=0.002 and *P*=0.001 respectively). By using haplotypes in the controls as references, the odds ratios for EH were estimated for haplotypes which were present at a significantly different frequency between the EH and control subjects, and the T-C haplotype established by rs3088283-rs1047303 showed a significantly high odds ratio of 1.702 (*P*=0.047).

Subsequently, we used the haplotypes that had the lowest overall *P* values to establish diplotypes (Table 5). The diplotype established by rs3088283-rs1047303 in the total group was associated with significantly higher SBP, DBP, and aldosterone levels in those subjects who had the T-C haplotype compared with those who did not (*P*=0.025, *P*=0.014, and *P*=0.006 respectively).

Hypertensive subjects who had one or two copies of the T-C haplotype were also found to have higher SBP and aldosterone levels compared to those lacking the T-C haplotype (*P*=0.031 and *P*=0.049 respectively). In males with the diplotype established by rs3088283-rs6203-rs1819698, both the SBP and aldosterone levels were significantly higher in subjects who had double T-C-G haplotypes compared with those who only had a single or who were completely lacking the T-C-G haplotype (*P*=0.012 and *P*=0.039 respectively). In females with the diplotype established by rs1047303-rs2854964, SBP and DBP were significantly higher in subjects who had the C-A haplotype compared with those who did not (*P*=0.005 and *P*=0.001 respectively). It should be noted, however, that these diplotypes had no association with echocardiographic parameters such as LVDD, IVST, PWTd, LVM or LVMI (data of PWTd and LVM, are not shown in Table 5). Furthermore, we investigated the effect of the T-C haplotype on the aldosterone level. We performed a logistic regression analysis that examined age, BMI, and the plasma renin activity and showed that the haplotype is associated with higher aldosterone level (>120 pg/ml; Table 6).

Discussion

The current study is the first to use haplotype and diplotype analyses in the same subjects in order to investigate the association between the *HSD3B1* gene and BP, aldosterone level, and echocardiographic

Table 5 Association between diplotypes and blood pressure, aldosterone level, and echocardiographic parameters.

Diplotypes	SBP (mmHg)	P value	DBP (mmHg)	P value	PAC (pg/ml)	P value	LVDd (mm)	P value	LVMi (g/m ²)	P value
Diplotypes established by rs3088283-rs1047303 haplotypes in total subjects										
NT+EH	One or two T-C	151.44±40.08	0.025	93.86±23.93	0.014	137.48±70.55	0.006	51.65±6.98	0.315	157.76±79.97
	haplotypes	(n=66)		(n=66)		(n=34)		(n=16)		(n=13)
	No T-C	141.35±33.45		86.91±21.30		107.24±56.47		49.90±6.33		130.00±53.79
	haplotypes	(n=495)		(n=495)		(n=206)		(n=94)		(n=86)
EH	One or two T-C	179.70±22.66	0.031	109.73±15.93	0.112	136.42±71.27	0.049	51.65±6.98	0.466	157.76±79.97
	haplotypes	(n=40)		(n=40)		(n=32)		(n=16)		(n=13)
	No T-C	172.18±19.87		105.96±13.42		114.10±55.59		50.33±6.41		139.12±55.95
	haplotypes	(n=235)		(n=235)		(n=160)		(n=71)		(n=66)
Diplotypes established by rs3088283-rs1819698 haplotypes in male subjects										
NT+EH	Two T-C-G	167.60±28.98	0.012	101.10±20.33	0.062	159.50±93.60	0.039	57.75±11.04	0.052	184.66±
	haplotypes	(n=10)		(n=10)		(n=7)		(n=4)		103.26 (n=4)
	No or one T-C-G	141.18±32.60		88.21±21.53		109.56±55.82		50.18±7.18		139.48±54.35
	haplotypes	(n=354)		(n=354)		(n=139)		(n=61)		(n=55)
Diplotypes established by rs1047303-rs2854964 haplotypes in female subjects										
NT+EH	One or two C-A	170.29±34.66	0.005	104.64±21.15	0.001	144.70±63.11	0.070	47.82±2.66	0.369	129.87±37.51
	haplotypes	(n=14)		(n=14)		(n=10)		(n=5)		(n=4)
	No C-A	141.67±36.82		84.77±21.42		107.46±60.57		49.651±4.36		120.27±57.41
	haplotypes	(n=183)		(n=183)		(n=85)		(n=40)		(n=37)

NT, normotension; EH, essential hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; PAC, plasma aldosterone concentration; LVDd, left ventricular diastolic dimension; LVMi, left ventricular mass index.

findings. The use of haplotypes and/or diplotypes is a more recent approach that may very well be used to elucidate relationships between candidate genes and specific traits. To avoid the possible confounding effect of ethnicity, we restricted our analysis to Japanese subjects. Since we selected our experimental population on the basis of a strong family history of HT, there is an increased likelihood that our findings of a genetic contribution to HT were associated with the inherent biological power of our patient cohort. Therefore, the positive results of this study might potentially be able to explain why conflicting results have been reported in the past when HT was used as the phenotype.

HSD3B is a key rate-limiting enzyme in the steroid biosynthesis pathways that produce aldosterone, estradiol, testosterone, and cortisol. It is expressed as two tissue-specific isoforms (HSD3B1 and HSD3B2) that have different substrate affinities (13). A genome-wide linkage analysis of 420 markers (353 microsatellites and 67 restriction fragment length polymorphisms) has shown that the locus for BP variations is within the region of the *HSD3B1* gene on chromosome 1p13.1 (14). Most recently, Doi *et al.* (5) have shown that Cry-null mice show salt-sensitive HT due to abnormally high synthesis of the mineralocorticoid aldosterone by the adrenal gland. In Cry-null mice, *Hsd3b6* mRNA and protein level are constitutively high, leading to a marked increase in Hsd3b activity and, as a consequence, enhanced aldosterone production. They also found that human *HSD3B1*, but not *HSB3B2*, is a functional counterpart of mouse *Hsd3b6*. In clinical examination of the human *HSD3B1* gene of Swedish NTs, a positive association between BP and rs6203 (the T→C Leu 338 variant of *HSD3B1*) was found with higher SBP and DBP occurring in carriers of the C allele (6). Conversely, a later study indicated that this SNP was not associated with HT in a Caucasian–Australian HT cohort, even though the study revealed that there was a tendency for higher DBP in those with the C allele (15). Our analysis, however, did confirm that the CC genotype of rs6203 was associated with higher BP in male but not in female subjects. As in a recent study (6), we also found that the aldosterone level tended to increase in accordance with the number of C alleles of rs6203 present. In addition, extremely high aldosterone levels were observed for the CC genotype of rs1047303 compared with the AA and the AC genotypes (data not shown). Although the reason for the gender difference was not clear, the potential mechanisms underlying the gender-related differences observed in our study merit comment. Both estrogen and aldosterone receptors are present in cardiac fibroblasts, myocyte, endothelial, and vascular smooth muscle cells. In addition, in vascular tissue, the rapid responses to aldosterone may be important in the acute control of BP (16). Aldosterone and estrogen also elicit similar rapid nongenomic responses that use common signaling pathways (protein kinase C), and some of the responses are shown to have gender

Table 6 Odds ratios and 95% CI for higher aldosterone level (> 120 pg/dl).

Risk factor	NT + EH (n=240)			EH (n=192)		
	Odds ratios	95% CI	P value	Odds ratios	95% CI	P value
T-C haplotype	2.302	1.040–5.096	0.040	5.408	1.580–18.508	0.007
Age	1.064	1.021–1.109	0.003	1.166	1.060–1.282	0.002
BMI	0.956	0.915–0.999	0.056	0.948	0.860–1.044	0.383
PRA	0.990	0.965–1.015	0.412	0.980	0.938–1.025	0.276

NT, normotension; EH, essential hypertension; CI, confidence intervals; BMI, body mass index; PRA; plasma renin activity. Haplotype was constructed with rs3088283 and rs1047303.

specificity (16). Because our present sample size was not sufficiently large to define gender differences, subsequent study is warranted to investigate whether an interaction between the signaling effect of estrogen and aldosterone receptors contributes to the observed gender-related differences in BP.

In animal models, both cardiac load and high circulating aldosterone levels can stimulate fibrosis within the myocardium, leading to LVH (17). Pathological patterns of the LV geometry have also been shown to be associated with an elevation of plasma aldosterone concentrations in the EH patients (18, 19). It has been estimated that the role of genetic factors in causing cardiac mass variance may be as high as 60% (20), and that these different gene variants are associated with LVH and diastolic dysfunction in EH. Heller *et al.* (21) assessed the influence of C344T aldosterone synthase polymorphism (*CYP11B2*) in left ventricular structure and humoral parameters in young NT males. They found that the TT genotype of the C344T polymorphism of the *CYP11B2* gene was associated with significantly higher plasma levels of aldosterone and higher values of the LVMI. On the other hand, a systematic review (22) of the association of the *CYP11B2* gene with echocardiographic parameters found that there was no significant association between the C344T polymorphism and LVM. Similarly, no significant association was found for interventricular septal wall thickness. However, the previous study did not examine the LVMI, which is an important parameter of LVH. In the present study, we evaluated the association between the *HSD3B1* gene and the LVMI, in addition to other echocardiographic parameters. However, we were also unable to reach a definitive conclusion with regard to the association between the diplotypes of *HSD3B1* and LVMI. One of the potentially limiting factors in the present study was the relatively small number of patients who underwent the echocardiographic examinations. Thus, the small sample size of our cohort may have limited the statistical power to detect weak associations between the diplotypes and the echocardiographic parameters. We also recognize that our analysis was limited in that we only examined six of the SNPs for the *HSD3B* genes, and that we did not investigate the *CYP11B1* and *CYP11B2* genes, which are key to the control of the

production of hormone during the late stages of aldosterone synthesis.

In concordance with a recent report (23), the findings for the tag SNPs and haplotype analysis in our study population further support the involvement of *HSD3B1* in BP regulation with rs6203 and rs1047303 both showing a definite association with BP. In contrast, our study indicates that the polymorphism within the *HSD3B2* gene, which is only 8.4 kb away from the *HSD3B1* gene, was not associated with BP. Thus, the observation that the *HSD3B1* gene is associated with increased BP and aldosterone levels suggests that a gain of function through a mutation in the *HSD3B1* gene is actually responsible for augmenting the biosynthesis of this hormone. In addition, recent studies have indicated that mutations of the enzymes involved in aldosterone biosynthesis can result in elevated or suppressed aldosterone levels (23, 24). Therefore, genetic polymorphisms such as rs1047303, which is a missense mutation of Thr 367 Asn in the exon of *HSD3B1*, might potentially modulate the exchange of the enzyme, thereby contributing to the inherited variability of the BP and aldosterone levels.

In conclusion, we employed a candidate-gene approach that uses a small set of highly informative SNPs to probe for significant associations with the BP and aldosterone levels. We observed a significant association between the *HSD3B1* variants and BP. Furthermore, the results of this study provide the first evidence that *HSD3B1* diplotypes variations not only have a significant association with BP but also with aldosterone level in the present subjects. Overall, our results reinforce the view that inherited variations in the steroid biosynthetic pathway could have effects on both the circulating aldosterone level and BP in the Japanese population. However, our present study only included Japanese subjects, and the sample size was too small to clearly confirm the associations between SNPs at the *HSD3B1* gene and EH and plasma aldosterone. A replication study with a larger and/or another population is indispensable to confirm our present results.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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