CLINICAL STUDY

Association of \textit{HSD3B1} and \textit{HSD3B2} gene polymorphisms with essential hypertension, aldosterone level, and left ventricular structure

Masanori Shimodaira\textsuperscript{1,2}, Tomohiro Nakayama\textsuperscript{1,3,4}, Naoyuki Sato\textsuperscript{3}, Noriko Aoi\textsuperscript{3,4}, Mikano Sato\textsuperscript{3,4}, Yoichi Izumi\textsuperscript{4}, Masayoshi Soma\textsuperscript{4,5} and Koichi Matsumoto\textsuperscript{4}

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

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

Abstract

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

\textbf{Methods:} We performed a haplotype- and diplotype-based case–control study to investigate the association between the \textit{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 \textit{HSD3B1} gene (rs3765945, rs3088283, rs6203, and rs1047303) and for two SNPs of the \textit{HSD3B2} gene (rs2854964 and rs1819698). Aldosterone and LVH were investigated in 240 and 110 subjects respectively.

\textbf{Results:} Significant differences were noted for the total and the male subject groups for the recessive model (CC versus TC \textit{C} \textit{T}) 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 \textit{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).

\textbf{Conclusion:} rs6203 and rs1047303 in the \textit{HSD3B1} gene are useful genetic markers for EH, while polymorphisms of \textit{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 \(\beta\)-hydroxysteroid dehydrogenases (\(\beta\)-HSD)/\(\Delta^4\)\textsuperscript{-isomerase} is the enzyme responsible for catalyzing the \(\beta\)-HSD dehydrogenation and \(\Delta^4\)\textsuperscript{-isomerization} of the \(\Delta^2\)-steroid precursors into their respective \(\Delta^4\)-ketosteroids (2). This activity is crucial for the synthesis of hormonal steroids, which includes aldosterone, cortisol, and testosterone. The two \(\beta\)-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 (\textit{HSD3B1}) is almost exclusively expressed as \(\beta\)-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 3β-HSD in the adrenal gland, ovary, and testis (4).

A recent study in mice shows that type VI 3β-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 (r² > 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](https://example.com/figure1.png)

**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, r² and pairwise LD (D') estimates are provided in the upper and lower triangles of each box respectively.
| Characteristics of the study participants. Continuous variables are expressed as means±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 (n=286)   | Males (n=184)   | Females (n=102) | Total (n=275)   | Males (n=180)   | Females (n=95)  |
| Age (years)     | 51.84±9.65      | 51.01±6.13      | 51.73±6.57      | 50.94±6.59      | 0.253           | 52.04±13.58     |
| BMI (kg/m²)     | 27.78±3.23      | 27.02±3.89      | 22.9±3.18       | 24.64±3.31      | <0.0001         | 22.49±3.33      |
| SBP (mmHg)      | 112.97±11.70    | 117.23±20.46    | 113.4±10.65     | 171.10±19.09    | <0.0001         | 112.93±13.41    |
| DBP (mmHg)      | 68.67±8.66      | 106.44±13.83    | 78.40±24.24     | 107.06±13.98    | <0.0001         | 68.25±23.23     |
| Pulse (beats/min) | 73.56±13.05     | 77.59±15.01     | 72.51±13.33     | 77.38±15.46     | 0.004           | 75.88±12.29     |
| Creatinine (mg/dl) | 0.83±0.22        | 0.85±0.25       | 0.90±0.20       | 0.94±0.24       | 0.162           | 0.71±0.18       |
| Tot. chol. (mg/dl) | 198.67±45.45     | 210.59±41.41    | 193.63±44.90    | 205.32±42.45    | 0.014           | 207.81±45.27    |
| HDL chol. (mg/dl) | 56.67±16.88      | 56.20±17.90     | 53.80±15.48     | 52.49±16.92     | 0.479           | 59.07±18.78     |
| Uric acid (mg/dl) | 5.37±1.48        | 5.68±1.60       | 5.82±1.42       | 6.20±1.47       | 0.016           | 4.55±1.20       |
| PAC (μg/ml)      | 106.34±55.44     | 117.82±58.87    | 108.24±45.79    | 115.58±59.31    | 0.590           | 104.87±62.75    |
| PRA (ng/ml per h) | 3.24±0.81        | 3.20±1.10       | 4.60±1.08       | 3.28±1.10       | 0.612           | 2.19±0.75       |
| ARR              | 126.81±126.73    | 172.23±267.69   | 116.94±159.29   | 155.14±262.63   | 0.320           | 134.49±96.84    |
| LVDd (mm)       | 47.56±6.01       | 50.57±6.50      | 48.96±6.01      | 50.57±6.50      | 0.183           | 47.12±3.31      |
| Hyperlipidemia (%) | 26.6             | 40.8            | 23.4            | 38.9            | 0.001           | 32.4            |
| Diabetes (%)    | 7.3              | 14.9            | 10.0            | 15.6            | 0.098           | 2.9             |
| Drinking (%)    | 38.8             | 62.2            | 48.4            | 76.1            | <0.0001         | 21.6            |
| Smoking (%)     | 28.0             | 51.3            | 35.9            | 62.2            | <0.0001         | 13.7            |

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.

To calculate the LVMI (g/m²), the LV was divided by the body surface area.

LVM = 0.81×1.044(LVDd + PWTD + IVST)^3/2

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

LVMI = LVM/BSA

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

Hyperlipidemia (%) = (Total Cholesterol + Triglycerides)/2

Diabetes (%) = (Systolic Blood Pressure + Diastolic Blood Pressure)/2

Drinking (%) = (SBP + DBP)/2

Smoking (%) = (SBP + DBP)/2

Biochemical analysis

Department methods employed by the Clinical Laboratory:

Echocardiographic examination

Standard method employed by the Clinical Laboratory.

Biochemical analysis

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Biochemical analysis

Department methods employed by the Clinical Laboratory.
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Allele frequencies are given as percentages. NT, normotension; EH, essential hypertension. N.C. indicates that the calculation of the $\chi^2$ 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 ± 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 markers for the HSD3B1 gene (rs3765945, rs3098823, 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 r² 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.

<table>
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<th>Risk factor</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>P value</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>P value</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>P value</th>
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<td>Female</td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
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<td>Odds ratios and 95% CI for hypertension risk factors and the CC genotypes of rs6203</td>
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<tr>
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<td>0.031</td>
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<td>0.420</td>
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<td>0.001</td>
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<td>&lt;0.001</td>
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<tr>
<td>Diabetes mellitus</td>
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<td>0.776</td>
<td>0.406–1.484</td>
<td>0.443</td>
<td>0.265</td>
<td>0.069–1.022</td>
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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 | 0.296   | 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.
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 haplotype established by rs3088283-rs1047303-rs2854964 was 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 only had a single or who were completely lacking the T-C-G haplotype. 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-G 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 diplotype had no association with echocardiographic parameters such as LVd, 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 echocardiographic parameters such as LVd, IVST, PWTd, LVM, or LVMi (data of PWTd and LVM, are not shown in Table 5).

**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 parameters.

<table>
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<th>Haplotype established by rs3088283-rs1047303 (Haplotype analysis in total subjects)</th>
<th>Frequency of the haplotype</th>
<th>Adjusted $P$ value</th>
<th>Odds ratio for EH (95% CI)</th>
<th>$P$ value</th>
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<th>Frequency of the haplotype</th>
<th>Adjusted $P$ value</th>
<th>Odds ratio for EH (95% CI)</th>
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</table>

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.
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.

**HSD3B1** 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 HSD3B2, 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–American 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 differences.

### Table 5

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

<table>
<thead>
<tr>
<th>Diplotypes</th>
<th>NT - EH</th>
<th>EH</th>
<th>NT - EH</th>
<th>EH</th>
<th>NT - EH</th>
<th>EH</th>
<th>NT - EH</th>
<th>EH</th>
<th>NT - EH</th>
<th>EH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP (mmHg)</td>
<td>P value</td>
<td>DBP (mmHg)</td>
<td>P value</td>
<td>LVDd (mm)</td>
<td>P value</td>
<td>LVMI (g/m²)</td>
<td>P value</td>
<td>PAC (pg/ml)</td>
<td>P value</td>
</tr>
<tr>
<td>N/T-C</td>
<td>141.35 ± 33.45</td>
<td>0.031</td>
<td>86.91 ± 21.30</td>
<td>0.011</td>
<td>68.60 ± 19.88</td>
<td>0.012</td>
<td>101.10 ± 20.33</td>
<td>0.082</td>
<td>153.92 ± 93.60</td>
<td>0.039</td>
</tr>
<tr>
<td>One or two T-C</td>
<td>179.70 ± 22.66</td>
<td>0.003</td>
<td>108.73 ± 16.93</td>
<td>0.012</td>
<td>138.42 ± 21.27</td>
<td>0.012</td>
<td>121.30 ± 20.33</td>
<td>0.082</td>
<td>157.77 ± 79.97</td>
<td>0.030</td>
</tr>
<tr>
<td>One or two T-C-G</td>
<td>141.86 ± 33.97</td>
<td>0.014</td>
<td>86.91 ± 21.30</td>
<td>0.011</td>
<td>68.60 ± 19.88</td>
<td>0.012</td>
<td>101.10 ± 20.33</td>
<td>0.082</td>
<td>153.92 ± 93.60</td>
<td>0.039</td>
</tr>
<tr>
<td>One or two C-A</td>
<td>179.70 ± 22.66</td>
<td>0.003</td>
<td>108.73 ± 16.93</td>
<td>0.012</td>
<td>138.42 ± 21.27</td>
<td>0.012</td>
<td>121.30 ± 20.33</td>
<td>0.082</td>
<td>157.77 ± 79.97</td>
<td>0.030</td>
</tr>
<tr>
<td>One or two C-A-G</td>
<td>141.86 ± 33.97</td>
<td>0.014</td>
<td>86.91 ± 21.30</td>
<td>0.011</td>
<td>68.60 ± 19.88</td>
<td>0.012</td>
<td>101.10 ± 20.33</td>
<td>0.082</td>
<td>153.92 ± 93.60</td>
<td>0.039</td>
</tr>
<tr>
<td>One or two C-A-T</td>
<td>179.70 ± 22.66</td>
<td>0.003</td>
<td>108.73 ± 16.93</td>
<td>0.012</td>
<td>138.42 ± 21.27</td>
<td>0.012</td>
<td>121.30 ± 20.33</td>
<td>0.082</td>
<td>157.77 ± 79.97</td>
<td>0.030</td>
</tr>
</tbody>
</table>
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 diplotype variations 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 diplotype 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 there is no definite association in 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 with HSD3B1 and EH and plasma aldosterone. We also recognize the 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.

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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 with HSD3B1 and EH and plasma aldosterone. Furthermore, the results of this study provide the first evidence that HSD3B1 diploidy 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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<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>P value</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>P value</th>
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<td>T-C haplotype</td>
<td>2.302</td>
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<td>0.040</td>
<td>5.408</td>
<td>1.580–18.508</td>
<td>0.007</td>
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<tr>
<td>Age</td>
<td>1.064</td>
<td>1.021–1.109</td>
<td>0.003</td>
<td>1.166</td>
<td>1.060–1.282</td>
<td>0.002</td>
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<tr>
<td>BMI</td>
<td>0.956</td>
<td>0.915–0.999</td>
<td>0.056</td>
<td>0.948</td>
<td>0.860–1.044</td>
<td>0.383</td>
</tr>
<tr>
<td>PRA</td>
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<td>0.965–1.015</td>
<td>0.412</td>
<td>0.980</td>
<td>0.938–1.025</td>
<td>0.276</td>
</tr>
</tbody>
</table>

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