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Research Paper

Association Study of Aromatase Gene (CYP19A1) in Essential Hypertension

Masanori Shimodaira[1], Tomohiro Nakayama[2], Naoyuki Sato[3], Kosuke Saito[2,4], Akihiko Morita[5], Ichiro Sato[6], Teruyuki Takahashi[7], Masayoshi Soma[8], Yoichi Izumi[8]

1. MD Program, Nihon University School of Medicine, Tokyo, Japan
2. Division of Receptor Biology, Advanced Medical Research Center, Tokyo, Japan
3. Division of Genomic Epidemiology and Clinical Trials, Advanced Medical Research Center, Tokyo, Japan
4. Department of Applied Chemistry, Toyo University School of Engineering, Tokyo, Japan
5. Department of Neurology, Division of Neurology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan
6. Department of Obstetrics and Gynecology, Nihon University School of Medicine, Tokyo, Japan
7. Department of Neurology, Graduate School of Medicine, Nihon University, Tokyo, Japan
8. Division of Nephrology and Endocrinology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan

Correspondence to: Tomohiro Nakayama, MD, Division of Receptor Biology, Advanced Medical Research Center, Nihon University School of Medicine, Ooyaguchi-kamimachi, 30-1 Itabashi-ku, Tokyo 173-8610, Japan. Tel: +81 3-3972-8111 (ext.8205); Fax: +81 3-5375-8076; E-mail: tnakayam@med.nihon-u.ac.jp

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Background: As aromatase-deficient mice, which are deficient in estrogens, reportedly have reduced blood pressure, the aromatase gene (CYP19A1) is thought to be a susceptibility gene for essential hypertension (EH). The aim of the present study was to investigate the relationship between CYP19A1 and EH by examining single nucleotide polymorphisms (SNPs).

Methods: Five SNPs in the human CYP19A1 gene (rs1870049, rs936306, rs700518, rs10046 and rs4646) were selected, and an association study was performed in 218 Japanese EH patients and 225 age-matched normotensive (NT) individuals.

Results: There were significant differences between these groups in the distribution of genotypes rs700518 and rs10046 in male subjects, and genotypes rs700518, rs10046 and rs4646 in female subjects. On multiple logistic regression analysis, a significant association between rs700518 (p=0.023) and rs10046 (p=0.036) in male subjects and rs700518 in female subjects (p=0.018) was noted. Interestingly, the risk genotypes of rs700518 and rs10046 showed a sex-dependent inverse relationship. Both SBP and DBP levels were higher in total (cases and controls) male subjects with the G/G genotype with rs700518 or the T/T genotype with rs10046 than in male subjects without the G/G genotype or T/T genotype. SBP levels were lower in female subjects with the G/G genotype with rs700518 than in female subjects without G/G. The A-T haplotype constructed with rs1870049 and rs10046 was a susceptibility marker for EH.

Conclusions: We confirmed that rs700518 and rs10046, as well as a haplotype constructed with rs1870049 and rs10046, in the human CYP19A1 gene can be used as genetic markers for gender-specific EH.

Key words: Essential hypertension, aromatase, CYP19A1, single nucleotide polymorphism, genetic

Introduction

High blood pressure or hypertension affects about 25% of adults and is an important risk factor for death from stroke, myocardial infarction and congestive heart failure. The main cause of hypertension is a primary condition known as essential hypertension (EH). EH is thought to be a multifactorial disease [1]. Several reports have indicated that there are susceptibility genes for EH, including those for estrogen, estrogen receptor [2] and aromatase [3]. The final stage of estrogen synthesis is catalyzed by aromatase.

There are numerous proposed mechanisms by which estrogen may bring about beneficial effects on the cardiovascular system. However, the precise role of estrogens has been difficult to establish, perhaps due to their wide variety of actions. In humans, estrogen facilitates vasodilation by stimulating prostacyclin and nitric oxide synthesis, as well as decreasing the production of vasoconstrictor substances, such as cyclooxgenase-derived products, reactive oxygen species, angiotensin II and endothelin-1 [4]. Estrogen also reduces the number of angiotensin type I (AT1) receptors [5]. Furthermore, men are at higher risk of developing cardiovascular disease than premenopausal women, and age-matched women have been shown to have lower blood pressure than men [6].

The aromatase enzyme complex catalyzes the

conversion of androgens to estrogens in a variety of tissues, including the ovary and placenta [7,8], brain [9] and adipose tissue [10]. It was recently demonstrated that both estrogens and aromatase are produced in vascular tissue, particularly in smooth muscle cells [11] and endothelial cells [12]. It has been reported that aromatase-deficient (ArKO) mice, which are deficient in estrogens due to deletion of the aromatase gene, exhibit reduced blood pressure (BP)[3]. Thus, we hypothesized that aromatase is one of the factors affecting BP, and that the aromatase gene is a susceptibility gene for hypertension, as single nucleotide polymorphisms (SNPs) in this gene are associated with differences in estrogen levels in human [13].

The human CYP19A1 gene, which encodes aromatase, consists of 503 amino acids and is located on chromosome 15q21.1 [14]. The gene is very unique; it contains 11 exons, with 9 exons being translated, interrupted by 10 introns (about 80 kb, exon 2a to exon 2), and consists of approximately 130 kilobase pairs (kb).

The aim of the present study was to investigate the relationship between the human CYP19A1 gene and EH by examining 5 SNPs in the human CYP19A1 gene (Figure 1) in Japanese individuals.

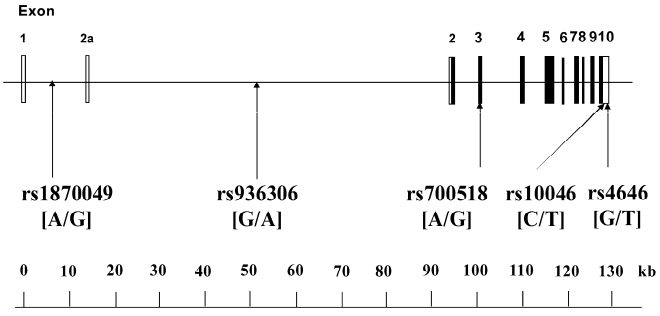

Figure 1. Organization of the human CYP19A1 gene and location of SNPs. The gene is approximately 130 kilobase pairs (kb) in length, and has a total of 11 exons. Boxes indicate exons, and lines indicate introns and intergenic regions. Filled boxes indicate coding regions. There are two transcript variants; variant 1 does not include exon 2a, and thus has a shorter 5'-UTR than transcript variant 2; variant 2 includes exon 2a. Both variants encode the same protein. Polymorphisms were expressed as nucleotide number on the sense strand of the CYP19A1 gene.

Subjects and Methods

Subjects

EH subjects were 218 patients diagnosed with EH according to the following criteria: seated systolic blood pressure (SBP) above 160 mmHg and/or diastolic blood pressure (DBP) above 100 mmHg, on 3 occasions within 2 months after the first medical examination. None of the EH subjects were using anti-hypertensive medication. Patients diagnosed with secondary hypertension were excluded. Control subjects were 225 healthy, normotensive (NT) individuals. None of the controls had a family history of hypertension, and they all had SBP and DBP below 130 and 85 mmHg, respectively. A family history of hypertension was defined as prior diagnosis of hypertension in grandparents, uncles, aunts, parents or siblings. Both groups were recruited from the northern area of Tokyo, Japan, and informed consent was obtained from each individual according to a protocol approved by the Human Studies Committee of Nihon University [15].

Biochemical analysis

Plasma concentration of total cholesterol, and serum concentrations of creatinine and uric acid were measured using the methods of the Clinical Laboratory Department of Nihon University Hospital [16].

Genotyping

Using information regarding allelic frequencies of SNPs registered with the National Center for Biotechnology Information (NCBI) and Celera Discovery System-Applied Biosystems, 5 SNPs with minor allele frequencies greater than 20% were selected. SNPs with relatively high minor allele frequencies have been shown to be useful as genetic markers for genetic association studies.

We selected 5 SNPs in the human CYP19A1 gene as markers for the genetic association experiment (Fig. 1). All 5 SNPs were confirmed using the NCBI website (accession numbers rs1870049, rs936306, rs700518, rs10046 and rs4646). rs1870049 and rs936306 are located in introns, rs700518 is a synonymous SNP that does not result in a change in amino acids, and rs10046 and rs4646 are located in the 3′-untranslated region. Genotypes were determined using Assays-on-Demand kits (Applied Biosystems, Branchburg, NJ) together with TaqMan® PCR. When allele-specific fluorogenic probes hybridize to the template during polymerase chain reaction (PCR), the 5'-nuclease activity of Taq polymerase is able to discriminate between alleles [17].

Linkage disequilibrium (LD) analysis and haplotype-based case-control analysis

LD analysis and haplotype-based case-control analysis were performed with SNPAlyze version 3.2.3 (Dynacom Co., Ltd., Yokohama, Japan) using 5 SNPs. The software is available from the following website: http://www.dynacom.co.jp/products/package/snpalyze/index.html. We used |D'| values of >0.5 to assign SNP locations to 1 haplotype block. SNPs with an r^2 value of <0.5 were selected as tagged. In the

haplotype-based case-control analysis, the frequency distribution of the haplotypes was calculated by performing a chi-squared test using the contingency table method.

Statistical analysis

Data are shown as means ±SD. Hardy-Weinberg equilibrium was assessed by chi-squared analysis in NT controls. The overall distribution of alleles was analyzed using 2 × 2 contingency tables, and the distribution of genotypes between EH patients and NT controls was tested using a 2-sided Fisher exact test and multiple logistic regression analysis, as the results of multiple logistic regression analyses after adjusting for confounding factors are known to be highly reliable. Statistical significance was established at $p < 0.05$. Differences in clinical data between the EH and NT groups were assessed by student t-test. Statistical analyses were performed using SPSS software for Windows, version 12 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the clinical features of the EH patients and NT controls. SBP, DBP, body mass index (BMI) and pulse rate were significantly higher in the EH group than in the NT group. Age, serum concentrations of creatinine, and plasma concentrations of total cholesterol and uric acid did not significantly differ between the two groups.

Table 1. Characteristics of study participants.

	Total				Men				Women			
	NT	EH	p Value		NT	EH	p Value		NT	EH	p Value	
Number of subjects	225	218			144	142			81	76		
Age (years)	50.3±9.01	50.5±6.2	0.812		50.0±5.6	50.2±6.7	0.809		50.9±13.1	51.1±5.3	0.899	
BMI (kg/m^2)	22.8±3.1	24.8±3.8	<0.001	*	23.0±2.9	24.8±3.6	<0.001	*	22.4±3.2	24.8±4.1	<0.001	*
SBP (mmHg)	111.8±11.0	173.1±19.0	<0.001	*	112.4±10.6	171.2±16.9	<0.001	*	110.9±11.6	176.7±22.0	<0.001	*
DBP (mmHg)	68.8±8.6	106.0±12.0	<0.001	*	69.6±8.2	106.8±10.9	<0.001	*	67.4±9.2	104.3±13.6	<0.001	*
Pulse (beats/min)	72.1±9.9	77.5±15.6	<0.001	*	76.9±9.5	77.8±16.0	<0.001	*	74.3±10.2	77.0±14.8	0.257	
Creatinine (mg/dl)	0.8±0.2	0.8±0.2	0.550		0.9±0.2	0.9±0.2	0.505		0.7±0.1	0.7±0.2	0.842	
Total cholesterol (mg/dl)	203.3±41.1	209.9±40.6	0.103		199.1±39.0	204.2±39.9	0.290		210.8±44.0	220.2±40.0	0.177	
Uric acid (mg/dl)	5.4±1.5	5.6±1.5	0.123		6.0±1.3	6.2±1.5	0.093		4.5±1.3	4.6±1.0	0.609	
Alcohol consumption (%)	70.5	69.0	0.761		77	85	0.084		38.8	37.7	0.904	
Smoking (%)	40.7	51.9	0.038	*	52.1	62.3	0.114		21.6	31.9	0.117	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; NT, normotension; EH, essential hypertension. *Significant difference

Table 2 shows the distribution of genotypic and allelic frequencies of the 5 SNPs in each group. The genotype distribution of the each SNP in NT controls did not differ significantly from the Hardy-Weinberg equilibrium values (data not shown). The overall distributions of genotype and allele frequencies of all 5 SNPs did not significantly differ between the EH and total NT groups. However, some distributions showed significant gender-based differences between the groups. Among men, there were significant differences between the EH and NT groups in the distribution of rs700518 (P=0.012) and rs10046 genotypes (P=0.005). In the dominant model, the G/G genotype was significantly more frequent than the A/A&A/G genotypes of rs700518 (P=0.009), and the T/T genotype was significantly more frequent than the C/C&C/T genotypes of rs10046 (P=0.003) in EH men. Furthermore, the genotype distribution showed reciprocal findings in women when compared to men; in EH women, the G/G genotype was significantly less frequent than the A/A&A/G genotypes of rs700518 (P=0.021), and the T/T genotype was significantly less frequent than the C/C&C/T genotypes of rs10046 (P=0.030). The T allele of SNP rs4646 (p=0.046) and the GT&T/T genotype (p=0.032) were significantly more frequent in EH women than in NT women.

Multiple logistic regression analysis revealed significant associations between rs700518 G/G and EH in men (p=0.023) and between rs10046 T/T and EH in men (p=0.036), even after adjustment for confounding factors such as age, BMI, creatinine, total cholesterol and uric acid. The calculated odds ratios were 2.48 (95%CI: 1.11-5.53) and 2.10 (95%CI: 1.04-4.23), respectively. Multiple logistic regression analysis revealed a significant association between rs700518 A/A&A/G and EH in women (p=0.018), even after adjustment for confounding factors such as age, BMI, creatinine, total cholesterol and uric acid. The calculated odds ratio was 3.31 (95%CI: 1.16-3.40). Multiple logistic regression analysis for rs10046 and rs4646 in women showed no significant associations (data not shown). The opposite direction of the association of rs700518 and rs10046 in men and women was confirmed by multiple logistic regression analysis (p=0.001, <0.001, respectively).

Table 2. Genotype and allele distributions among NT subjects and patients with EH.

		Total subjects							Men							Women						
		NT		EH		chi-square p Value	Odss ratio	95%CI	NT		EH		chi-square p Value	Odss ratio	95%CI	NT		EH		chi-square p Value	Odss ratio	95%CI
Number of paticipants		225		218					144		142					81		76				
Variants																						
rs1870049	Genotype																					
	A/A	144	0.640	134	0.615				89	0.618	87	0.613				55	0.679	47	0.618			
	A/G	72	0.320	75	0.344				48	0.333	50	0.352				24	0.296	25	0.329			
	G/G	9	0.040	9	0.041	0.856	1.11	0.76-1.64	7	0.049	5	0.035	0.826	1.40	0.43-4.52	2	0.025	4	0.053	0.561	2.19	0.39-12.3
	Allele																					
	A	360	0.800	343	0.787				224	0.783	224	0.789				134	0.827	119	0.783			
	G	90	0.200	93	0.213	0.625	1.08	0.78-1.50	62	0.217	60	0.211	0.907	1.03	0.69-1.54	28	0.173	33	0.217	0.322	1.32	0.76-2.33
rs936306	Genotype																					
	G/G	88	0.391	89	0.408				56	0.392	60	0.423				31	0.383	29	0.382			
	G/A	102	0.453	104	0.477				63	0.441	66	0.465				39	0.481	38	0.500			
	A/A	35	0.156	25	0.115	0.454			24	0.168	16	0.113	0.420			11	0.136	9	0.118	0.942		
	Allele																					
	G	278	0.618	282	0.647				177	0.615	186	0.655				101	0.623	96	0.632			
	A	172	0.382	154	0.353	0.371	1.13	0.86-1.49	111	0.385	98	0.345	0.316	1.19	0.85-1.67	61	0.377	56	0.368	0.882	1.04	0.66-1.64
rs700518	Genotype																					
	A/A	82	0.364	88	0.404				55	0.382	58	0.408				27	0.333	30	0.395			
	A/G	111	0.493	95	0.436				77	0.535	57	0.401				34	0.420	38	0.500			
	G/G	32	0.142	35	0.161	0.478	1.18	0.80-1.73	12	0.083	27	0.190	0.012 *	2.58	1.25-5.33 * #	20	0.247	8	0.105	0.068	2.79	1.14-6.79 * #
	Allele																					
	A	275	0.611	271	0.622				187	0.649	173	0.609				88	0.543	98	0.645			
	G	175	0.389	165	0.378	0.749	1.05	0.80-1.37	101	0.351	111	0.391	0.320	1.19	0.85-1.67	74	0.457	54	0.355	0.067	1.53	0.97-2.40
rs10046	Genotype																					
	C/C	66	0.293	66	0.303				44	0.306	46	0.324				22	0.272	20	0.263			
	C/T	116	0.516	103	0.472				83	0.576	60	0.423				33	0.407	43	0.566			
	T/T	43	0.191	49	0.225	0.591	1.23	0.77-1.94	17	0.118	36	0.254	0.005 *	2.54	1.35-4.77 * #	26	0.321	13	0.171	0.061	2.29	1.07-4.89 *
	Allele																					
	C	248	0.551	235	0.539				171	0.594	152	0.535				77	0.475	83	0.546			
	T	202	0.449	201	0.461	0.717	1.05	0.81-1.37	117	0.406	132	0.465	0.158	1.27	0.91-1.77	85	0.525	69	0.454	0.210	1.32	0.85-2.07
rs4646	Genotype																					
	G/G	119	0.529	114	0.523				71	0.493	82	0.577				48	0.593	32	0.421			
	G/T	85	0.378	83	0.381				55	0.382	44	0.310				30	0.370	39	0.513			
	T/T	21	0.093	21	0.096	0.990	1.02	0.71-1.49	18	0.125	16	0.113	0.347	1.41	0.88-2.24	3	0.037	5	0.066	0.094	2.00	1.06-3.78 *
	Allele																					
	G	323	0.718	311	0.713				197	0.684	208	0.732				126	0.778	103	0.678			
	T	127	0.282	125	0.287	0.883	1.02	0.76-1.37	91	0.316	76	0.268	0.203	1.26	0.88-1.81	36	0.222	49	0.322	0.046 *	1.67	1.01-2.75 *

NT, normotension; EH, essential hypertension.
95%CI, 95% confidence interval
Odds ratios and 95%CIs were calculated as the risks of the susceptibility allele or genotype(s) for EH.
*Significant differences by chi-square analysis
#Significant differences by multiple logistic regression analysis

Clinical characteristics of the study participants by genotype are shown in Table 3. Genotypes showing significant differences in distribution on multiple logistic regression analysis were selected for analysis. Both SBP and DBP levels were higher in total (EH plus NT) male subjects with the G/G genotype in rs700518 than in male subjects without the G/G genotype. Furthermore, both SBP and DBP levels were higher in total male subjects with the T/T genotype in rs10046 than in male subjects without the T/T genotype. In contrast, SBP levels were higher in total female subjects with the A/A&A/G genotype in rs700518than in female subjects without the A/A&A/G genotype.

LD patterns in the CYP19A1 gene are illustrated by their |D′| values in NT groups (Table 4). The |D'| values indicate that all 5 SNPs are located in 1 haplotype block, as most |D'| values were over 0.5, except for rs1870049-rs700518, rs1870049-rs10046 and rs936306-rs10046. All pair-wise SNPs, except rs700518-rs10046, were available for the performance of a haplotype-based case-control study because all r^2 values were below 0.5. Because r^2 values calculated for the rs700518 and rs10046 SNPs were large, we did not perform a haplotype-based association study using the 2 SNPs in the same analysis. All 18 combinations of pair-wise SNPs were analyzed in men and women. Significant differences in overall distribution were only seen for the rs1870049 and rs10046 combination in men. Thus, the A-C haplotype is a resistance marker for EH, while the A-T haplotype is a susceptibility marker for EH. There is no overall distribution showing a significant difference in women (Table 5).

Table 3. Clinical characteristics of the study partipants in each genotype.

Men

	rs700518				rs10046			
	A/A&A/G	G/G	p Value		C/C&C/T	T/T	p Value	
Number of subjects	247	39			233	53		
Age (years)	50.2±6.1	49.7±6.1	0.666		50.1±6.2	49.9±5.8	0.812	
BMI (kg/m^2)	23.9±3.5	24.1±3.1	0.820		23.9±3.5	24.2±3.1	0.511	
SBP (mmHg)	139.4±32.1	155.6±33.1	0.004	*	138.7±32.1	154.2±32.5	0.002	*
DBP (mmHg)	86.7±20.8	96.3±20.2	0.008	*	86.3±20.7	95.5±20.8	0.004	*
Pulse (beats/min)	75.3±14.6	73.1±11.0	0.453		75.3±15.0	73.7±10.1	0.522	
Creatinine (mg/dl)	0.9±0.2	1.0±0.2	0.193		0.9±0.2	0.9±0.2	0.262	
Total cholesterol (mg/d	202.2±39.6	197.9±38.3	0.557		201.0±39.7	204.6±38.4	0.555	
Uric acid (mg/dl)	6.1±1.4	6.0±1.4	0.795		6.1±1.4	6.0±1.3	0.835	
Alcohol consumption ('	82.8	76.7	0.415		82.2	81.0	0.848	
Smoking (%)	59.3	44.1	0.819		60.0	51.1	0.383	

Table 4. Pairwise LD in CYP19A1 gene of each NT group.

SNP	rs1870049	rs936306	rs700518	rs10046	rs4646
rs1870049		0.934	0.176	0.257	0.653
rs936306	0.352		0.567	0.384	0.764
rs700518	0.005	0.127		0.967	1.000
rs10046	0.013	0.074	0.730		0.976
rs4646	0.042	0.142	0.250	0.305	

LD, linkage disequilibrium; SNP, single-nucleotide polymorphism.
Upper right triangle shows absolute D' values. D' > 0.5 are shown as shaded values.
Lower left triangle shows r^2 values. r^2 > 0.5 are shown as shaded values.

Table 5. Haplotypes showing significant differences in overall distribution between NT controls and EH patients in men.

Combination of SNPs	Overall distribution		Distribution of Individual haplotypes					
	Chi-square	p value	Haplotype	NT 288	EH 284	Chi-square	p-value	
rs1870049-rs10046	8.1	0.044	A-C	0.464	0.381	4.232	0.040	*
			A-T	0.320	0.408	4.895	0.027	*
			G-C	0.129	0.155	0.823	0.364	
			G-T	0.086	0.057	1.995	0.158	

* significant difference

Discussion

Human aromatase deficiency was first reported in 1995. The disorder is very rare, and only a few cases have been reported [18-20]. Male patients with aromatase deficiency exhibit eunuchoid skeletal proportions, macroorchidism, sexually precocity. In contrast, female patients with the disease develop progressive signs of virilization, pubertal failure with no signs of estrogen action, hypergonadotropic hypogonadism, polycystic ovaries on pelvic sonography, and tall stature. Common clinical data in men and women with aromatase deficiency are high levels of plasma testosterone, androsterone, FSH and LH, and low estradiol and estrone [18,19]. They also have homozygous or compound heterozygous mutations in the CYP19A1 gene. Interestingly, male patients with aromatase deficiency exhibit hypertension [19,20].

In the present study, the findings regarding genotype and allele distributions were particularly interesting from the viewpoint of gender differences. The gender differences in genotype and allele distributions were similar between rs700518 and rs10046, while the overall distribution of genotypes was significantly different between the EH and the NT groups. Blood pressure values for each genotype were

similar between rs700518 and rs10046. These results were consistent with those of LD analysis showing that rs700518 and rs10046 were closely linked with a large r^2.

Although systolic BP in ArKO female mice was similar to that in age- and weight-matched wild-type (WT) mice, diastolic and mean BP were lower in ArKO mice (-6.3 ± 1.9 and -4.6 ± 2.1 mmHg, respectively). The baroreflex sensitivity of ArKO mice was 46% that observed in WT mice [3]. However, there have been no previous studies on male ArKO mice or comparing data between male and female ArKO mice.

Some investigators have been reported the CYP19A1 gene variants associated with hypertension. Peter et al. found suggestive evidence of gender-specific contributions of rs4646 to DBP variation in women in the Framingham Heart Study [21]. DBP in patients with T/T genotype was significantly higher than in those without this genotype. This is very interesting because the frequencies of EH women with T/T genotype or T alleles were significantly higher in the present study when compared to NT women. In addition, our data for rs4646 also showed no significant results in men, which is also in agreement the report by Peter et al. Recently, Ramirez-Lorca et al. reported that DBP in subjects with C/C genotype in rs10046 was significantly higher than in those without C/C genotype [22]. This corresponds with our data, as the frequency of EH patients with the T/T genotype was significantly lower than that of NT subjects. However, the opposite direction of the association in men found in our study was not detected in men in their study. There are several reasons for this discrepancy between the results in our study and those of previous studies. Our study used a case-control design with patients clearly diagnosed by EH criteria, while Ramirez-Lorca et al. used a population-based cohort in the general population. Therefore, the data on blood pressure in each genotype from their study were within normal ranges. This discrepancy may be attributed to both the different criteria used in subject selection, and to racial differences in the populations studied.

In the present study, none of the SNPs were thought to have functional consequences. Possible functional mutations in the CYP19A1 gene with quantitative effects on genomic transcription, posttranslational processing or amino acid sequence have a strong linkage with genetic markers such as rs10046, and subsequently reduce the activity of aromatase associated with EH. Unfortunately, we were not able to obtain samples to measure plasma sex hormones levels and aromatase activity, due to the difficulty in obtaining written informed consent for blood examinations from subjects not receiving medications.

In conclusion, the present study was the first to examine correlations between the human CYP19A1 gene (encoding aromatase) and EH. The present data indicate that the CYP19A1 gene is a gender-specific candidate genetic marker for EH.

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Conflict of interest

The authors have declared that no conflict of interest exists.

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