Introduction

The endothelin (EDN) system is comprised of 4 active EDNs, with EDN1 being the predominant isoform in the cardiovascular system (*1*). Because of the potent vasoconstricting and mitogenic effects of EDN1 and its involvement in various cardiovascular diseases, biosynthesis of EDN1 has received considerable attention. EDN1 is synthesized from a 212-amino acid precursor protein, preproEDN1, through multiple proteolytic steps. In the first step, preproEDN1 is cleaved by a signal peptidase, resulting in the formation of proEDN1, which is then cleaved by a furin-like enzyme to yield the 38-amino acid protein known as big-EDN1 (amino acids 53–92) or other intermediates. Big-EDN1 is subsequently cleaved by a unique type II metalloprotease, EDN-converting enzyme-1 (ECE1), to yield EDN1 (amino acids 53–73) (*2*).

The EDN system is a promising target for the genetic analysis for hypertension. The missense mutation Lys198Asn has been identified in preproEDN1, and several reports have described that this polymorphism showed a positive association with blood pressure elevation in overweight people (*3–5*), although no significant difference in the EDN1 levels between the Asn-type and Lys-type transfectant was observed in an expression analysis (*6*). As for *ECE1*, an association between the −338C>A polymorphism in *ECE1* and blood pressure levels in women but not in men has recently been reported (*7*). This C>A polymorphism is associated with increased promoter activity, as demonstrated in a promoter assay analysis (*8*).

Complex traits such as hypertension, diabetes mellitus, and hyperlipidemia are suggested to be caused by common sequence variants that may have a small to moderate phenotypic effect (*9–11*). On the other hand, accumulating data has shown that most Mendelian disorders are caused by a set of different mutations that often reside in coding regions. These rare variants tend to have strong phenotypic effects. Several recent studies have shown that rare genetic variations in *ABCA1*, *APOA1*, and *LCAT* collectively contribute to the variation in plasma levels of high-density lipoprotein (HDL) cholesterol in the general population (*12*, *13*). We hypothesized that rare genetic variations in hypertension candidate genes could collectively contribute to hypertension. To investigate this hypothesis, we have been identifying such mutations in Japanese hypertensive subjects; to date, we have identified missense mutations in the β- or γ-subunit of the amiloride-sensitive epithelial sodium channel encoded by *SCNN1B* and *SCNN1G* (*14*), a causative gene for pseudohypoaldosteronism type II encoded by serine-threonine kinase *WNK4* (*15*), the regulator of G-protein signaling 2 (*RGS2*) (*16*), and the mineralocorticoid receptor encoded by *NR3C2* (*17*). As the next hypertension candidate gene, we have begun to sequence the *EDN1* gene and to search for missense mutations (*18*).

In present study, we genotyped the genetic polymorphisms of one of the EDN-converting enzymes, the *ECE1* gene, in a general Japanese population to examine whether the *ECE1* gene is a susceptibility gene for hypertension. Secondly, to evaluate the EDN system in essential hypertension in Japanese, we re-sequenced the EDN1 polypeptide in the *EDN1* gene in Japanese hypertensive patients to identify missense mutations that may deleteriously affect EDN1 function.

Methods

General Population

The selection criteria and design of the Suita study have been described previously (*19*, *20*). Only those who gave written informed consent for genetic analyses were included in this study. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center. In this study, the genotypes of 1,873 samples were determined. The characteristics of the 1,873 participants (863 men and 1,010 women) are shown in Table 1. Routine blood examinations that included total serum cholesterol, HDL cholesterol, triglyceride, and glucose levels were performed. A physician or nurse interviewed each patient regarding smoking and alcohol drinking habits and personal history of cardiovascular disease, including angina pectoris, myocardial infarction, and/or stroke. Blood pressure was measured after at least 10 min of rest in a sitting position. Systolic and diastolic blood pressures (SBP and DBP) were the means of two measurements by well-trained doctors (recorded >3 min apart). Hypertension was defined as SBP of ≥140 mmHg, DBP of ≥90 mmHg, or the current use of antihypertensive medication (*20*). Diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/L (126 mg/dL), non-fasting plasma glucose ≥11.1 mmol/L (200 mg/dL), current use of antidiabetic medication, or HbA1c ≥6.5%. Hyperlipidemia was defined as total cholesterol ≥5.68 mmol/L (220 mg/dL) or antihyperlipidemia medication. Body mass index (BMI) was calculated as weight (in kg) divided by height (in m) squared.

Hypertensive Subjects

A total of 942 hypertensive subjects (518 men and 424 women; average age: 65.1±10.5 years) were recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center. Ninety-two percent of study subjects (870 subjects) were diagnosed with essential hypertension, and the rest had secondary hypertension, including renal hypertension (36 subjects), renovascular hypertension (23 subjects), primary aldosteronism (11 subjects) and hypothyroid-induced hypertension (2 subjects) (*14–17*). The hypertension criteria were blood pressure above 140 and/or 90 mmHg or the use of antihypertensive agents. Blood pressure was the average of three measurements taken in a sitting position after at least 5 min of rest on each occasion. About one-third of the hypertensive subjects had hypertensive cardiovas-

Table 1. Basic Characteristics of Subjects in Japanese General Population (Suita Study)

	Women (n=1,010)	Men (n=863)
Age (yeasr old)	63.3±11.0	66.3±11.1*
Systolic blood pressure (mmHg)	128.0±19.6	131.9±19.5*
Diastolic blood pressure (mmHg)	76.6±9.8	79.7±10.7*
Body mass index (kg/m^2)	22.3±3.2	23.3±3.0*
Total cholesterol (mmol/L)	5.57±0.79*	5.10±0.78
HDL-cholesterol (mmol/L)	1.67±0.40*	1.42±0.36
Current smokers (%)	6.3	30.1†
Current drinkers (%)	29.3	67.0†
Present illness (%)		
Hypertension	38.2	47.4†
Hyperlipidemia	55.2†	27.4
Diabetes mellitus	5.2	12.6†

Values are mean±SD or percentage. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dL or antihyperlipidemia medication; diabetes: fasting plasma glucose ≥126 mg/dL or non-fasting plasma glucose ≥200 mg/dL or HbA1c ≥6.5% or antidiabetic medication. *$p<0.05$ between women and men by Student's t-test. †$p<0.05$ between women and men by χ^2 test. HDL, high-density lipoprotein.

cular complications. The clinical features of the patients in this study are summarized in Table 2.

All of the participants for the genetic analysis in the present study gave their written informed consent. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Genotyping of Mutations of Single Nucleotide Polymorphisms of the *ECE1* Gene in the General Population

We obtained genetic polymorphisms in the *ECE1* gene using the database of Japanese Single Nucleotide Polymorphisms (JSNP) (http://snp.ims.u-tokyo.ac.jp/) (*21*, *22*) and genotyped the following 5 single nucleotide polymorphisms (SNPs) by the TaqMan-PCR system: rs212548-TC (IMS-JST017298 in intron 4), rs212528-TC (IMS-JST004319 in intron 5), rs212526-CT (IMS-JST009090 in intron 6), rs2038090-AC (IMS-JST004325 in intron 17), and rs2038089-AG (IMS-JST004324 in intron 17). The primers and probes of the TaqMan-PCR system are available on request. Hereafter, SNPs are described according to the RS nomenclature system.

Screening of Mutations in Exon 2 of the *EDN1* Gene

Blood samples were obtained from each subject and genomic DNA was isolated from peripheral blood leukocytes using an NA-3000 nucleic acid isolation system (KURABO, Osaka, Japan). The region of exon 2 was amplified by polymerase chain reaction (PCR) using a pair of specific primers, 5′-CTGATGGCAGGCTGTGTGCTT-3′ and 5′-CCCCATCAG ATGCCACTGTGA-3′, which flank the 612-bp region containing exon 2. The PCR products were directly sequenced on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA) as described previously (*23*, *24*). The obtained sequences were examined for the presence of mutations using Sequencher software (Gene Codes Corporation, Ann Arbor, USA), followed by visual inspection (*25*).

Table 2. General Characteristics of Patients with Hypertension and/or Renal Failure

Number	942
Age (years)	65.1±10.5
Gender (M/F)	518/424
Body mass index (kg/m^2)	24.2±3.3
Systolic blood pressure (mmHg)	145.5±19.2
Diastolic blood pressure (mmHg)	84.8±13.4
Essential hypertension	870
Secondary hypertension	72
Renal hypertension	36
Renovascular hypertension	23
Primary aldosteronism	11
Hypothyroid-induced hypertension	2
Renal impairment*	110
Ischemic heart disease	102
Stroke**	145

Values are expressed as mean±SD. *Patients who had serum creatinine ≥1.4 mg/dL. **Silent cerebral infarction was included. M, male; F, female.

Statistical Analysis

Analysis of variance was used to compare mean values between groups, and if overall significance was demonstrated, the intergroup difference was assessed by means of a general linear model. Frequencies were compared by χ^2 analysis. Association analyses between genotypes and blood pressure in each sex were performed through logistic regression analysis with consideration for potential confounding risk variables, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive medication. For multivariate risk predictors, the adjusted odds ratios were given with the 95% confidence intervals. The relationship between genotypes and risk of hypertension was expressed in terms of the odds ratios adjusted for possible confounding effects, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking). Odds ratios were calculated as a measure of the association between each genotype

Table 3. Odds Ratio of Polymorphisms in *ECE1*

SNP	Sex	Genotype	*n*	Odds ratio	(95% CI)	*p*	Genotype	*n*	Odds ratio	(95% CI)	*p*
rs212548	Women	TT	328	1	(reference)		TT+TC	821	1	(reference)	
		TC+CC	686	1.28	(0.94–1.74)	0.116	CC	193	1.21	(0.85–1.72)	0.293
	Men	TT	275	1	(reference)		TT+TC	692	1	(reference)	
		TC+CC	590	1.10	(0.82–1.50)	0.520	CC	173	0.98	(0.69–1.40)	0.924
rs212528	Women	TT	663	1	(reference)		TT+TC	980	1	(reference)	
		TC+CC	347	1.40	(1.04–1.89)	0.026	CC	30	1.63	(0.74–3.58)	0.227
	Men	TT	528	1	(reference)		TT+TC	827	1	(reference)	
		TC+CC	335	0.83	(0.62–1.11)	0.198	CC	36	0.75	(0.37–1.53)	0.428
rs212526	Women	CC	734	1	(reference)		CC+CT	996	1	(reference)	
		CT+TT	280	0.76	(0.55–1.05)	0.099	TT	18	0.77	(0.25–2.35)	0.650
	Men	CC	615	1	(reference)		CC+CT	842	1	(reference)	
		CT+TT	251	0.95	(0.70–1.30)	0.751	TT	24	1.40	(0.58–3.38)	0.455
rs2038090	Women	AA	774	1	(reference)		AA+AC	999	1	(reference)	
		AC+CC	239	1.17	(0.84–1.64)	0.348	CC	14	1.05	(0.30–3.61)	0.939
	Men	AA	676	1	(reference)		AA+AC	856	1	(reference)	
		AC+CC	189	1.00	(0.71–1.40)	0.989	CC	9	3.32	(0.67–16.45)	0.142
rs2038089	Women	AA	414	1	(reference)		AA+AG	880	1	(reference)	
		AG+GG	598	1.19	(0.89–1.59)	0.240	GG	132	1.21	(0.80–1.84)	0.358
	Men	AA	380	1	(reference)		AA+AG	788	1	(reference)	
		AG+GG	486	1.12	(0.84–1.49)	0.450	GG	78	1.33	(0.81–2.18)	0.264

*Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking). SNP, single nucleotide polymorphism; CI, confidence interval.

Table 4. Association of Genotypes with Blood Pressure Variation

SNP	Genotype	Women					Men				
		n	DBP (mmHg)	*p**	SBP (mmHg)	*p**	*n*	DBP (mmHg)	*p**	SBP (mmHg)	*p**
rs212528	TT	663	76.49±0.37		126.89±0.64		528	79.98±0.43		131.94±0.75	
	TC	317	76.55±0.53		129.21±0.93		299	79.48±0.57		131.18±1.00	
	CC	30	77.57±1.72	0.698	133.33±3.02	0.007	36	80.93±1.66	0.931	133.83±2.89	0.941
	TT	663	76.49±0.37		126.89±0.64		528	79.98±0.43		131.94±0.75	
	TC+CC	347	76.63±0.51	0.823	129.56±0.89	0.016	335	79.64±0.54	0.840	131.47±0.94	0.698
	TT+TC	980	76.51±0.30		127.64±0.53		827	79.67±0.34		131.66±0.60	
	CC	30	77.57±1.72	0.545	133.33±3.02	0.066	36	80.93±1.66	0.458	133.83±2.89	0.463
rs212526	CC	734	76.56±0.35		128.07±0.61		615	79.41±0.40		131.67±0.69	
	CT	262	76.90±0.58		127.51±1.03		227	80.15±0.66		131.39±1.15	
	TT	18	70.08±2.19	0.344	120.04±3.87	0.175	24	84.13±2.06	0.048	138.16±3.59	0.422
	CC	734	76.56±0.35		128.07±0.61		615	79.41±0.40		131.67±0.69	
	CT+TT	280	76.45±0.56	0.874	127.02±0.99	0.371	251	80.52±0.63	0.135	132.03±1.09	0.780
	CC+CT	996	76.65±0.30		127.92±0.52		842	79.61±0.34		131.59±0.59	
	TT	18	70.08±2.19	0.003	120.04±3.87	0.044	24	84.13±2.06	0.030	138.16±3.59	0.071

Values are mean±SEM. *Conditional logistic analysis, adjusted for age, body mass index (BMI), present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking). SNP, single nucleotide polymorphism; DBP, diastolic blood pressure; SBP, systolic blood pressure.

and hypertension under the assumption of a dominant (with scores of 0 for patients homozygous for the major allele and 1 for carriers of the minor allele) or recessive (with scores of 0 for carriers of the major allele and 1 for patients homozygous

Table 5. Haplotype Frequency (Freq) of *ECE1* Gene in Hypertensives (HT) and Normotensives (NT)

Haplotype		All				Men				Women			
		Freq (%)	χ^2	*p* Asymptotic	*p* Permutation	Freq (%)	χ^2	*p* Asymptotic	*p* Permutation	Freq (%)	χ^2	*p* Asymptotic	*p* Permutation
H1 T/T/C/A/A	Overall	19.2	1.278	0.258	0.327	19.0	0.040	0.841	0.893	19.3	2.954	0.086	0.127
	NT	19.8				18.8				20.4			
	HT	18.4				19.2				17.3			
H2 C/C/C/A/A	Overall	16.2	1.305	0.253	0.284	17.5	0.193	0.661	0.669	15.1	2.991	0.084	0.091
	NT	15.5				17.9				14.0			
	HT	16.9				17.1				16.9			
H3 T/T/C/A/G	Overall	14.3	0.122	0.727	0.769	14.7	0.231	0.631	0.695	14.2	0.060	0.807	0.825
	NT	14.1				14.4				14.0			
	HT	14.5				15.2				14.4			
H4 C/T/C/A/A	Overall	11.8	0.181	0.670	0.716	11.9	0.033	0.857	0.867	11.8	0.250	0.617	0.699
	NT	12.0				12.1				12.1			
	HT	11.5				11.8				11.4			
H5 T/T/T/A/A	Overall	10.7	8.254	0.004	0.015	10.9	0.421	0.516	0.575	10.6	11.865	0.001	0.003
	NT	12.0				11.4				12.4			
	HT	9.0				10.4				7.5			
H6 T/T/C/C/G	Overall	8.3	0.317	0.574	0.618	7.8	0.327	0.568	0.624	9.0	0.001	0.974	0.978
	NT	8.1				8.1				9.0			
	HT	8.7				7.3				9.0			
H7 C/T/C/A/G	Overall	7.8	0.133	0.715	0.775	6.2	1.115	0.291	0.402	8.8	2.071	0.150	0.192
	NT	7.6				5.5				8.2			
	HT	7.9				6.7				10.0			

Haplotypes (rs212548/rs212528/rs212526/rs2038090/rs2038089) with frequencies of more than 5% are shown. One hundred thousand replicates were used for permutation test for all, men and women. Numbers of haplotypes in Overall, NT, and HT are 3,736, 2,150, 1,586 for All; 1,730, 914, 816 for men; and 2,030, 1,254, 776 for women, respectively.

for the minor allele) mode of inheritance. The *p* values were adjusted by Bonferroni correction. SAS statistical software (release 6.12; SAS Institute Inc., Cary, USA) was used for the statistical analyses. The data of linkage disequilibrium, haplotype blocks and coverage of HapMap SNPs were downloaded from the HapMap Consortium (http://www.hapmap.org). Haplotypes and permutation analyses were calculated using SNPAlyze version 4.0 software (DYNACOM Co., Mobara, Japan).

Results

Association between SNPs in the *ECE1* Gene and Hypertension

Five genetic polymorphisms in the *ECE1* gene were genotyped in 1,873 individuals. The genotype frequencies for each polymorphism were as follows: rs212548-T>C, 0.563/0.437; rs212528-T>C, 0.800/0.200; rs212526-C>T, 0848/0.152; rs2038090-A>C, 0.880/0.120; rs2038089-A>G, 0.655/0.345. None of the genotype frequencies were significantly different from those expected from the Hardy-Weinberg equilibrium ($p>0.05$). Multiple logistic regression analysis after adjusting for confounding factors of age, BMI, hyperlipidemia, diabetes mellitus, smoking, and drinking revealed that one polymorphism, rs212528, in intron 5 was significantly associated with hypertension in women (rs212528-T>C: TT *vs.* TC+CC; odds ratio=1.40; 95% confidence interval: 1.04–1.89; $p=0.026$) (Table 3). The SBPs in women with the TT, TC, and CC genotypes were 126.89±0.64 mmHg ($n=663$), 129.21±0.93 mmHg ($n=317$), and 133.33±3.02 mmHg ($n=30$) ($p=0.007$), after adjusting for the same confounding factors (Table 4). Thus, the difference in SBP was 6.44 mmHg between women with the CC genotype and those with the TT genotype. This association was still significant even after the Bonferroni correction.

Another polymorphism, rs212526, was associated with a significant difference in DBP: women having the CC+CT genotype had a DBP of 76.65±0.30 mmHg ($n=996$) and those with the TT genotype had a DBP of 70.08±2.19 mmHg ($n=18$) ($p=0.003$) after adjusting for the same confounding factors (Table 4). This polymorphism was also significantly associated with the SBP in women (CC+CT: 127.92±0.52 mmHg, $n=996$; TT: 120.04±3.87 mmHg, $n=18$; $p=0.044$). However, this polymorphism did not show a significant association with hypertension. In men, this polymorphism was

Table 6. List of 5 Polymorphisms and Their Allele Frequency in Exon 2 of *EDN1* Identified by Direct Sequencing of 942 Hypertensive Japanese

Allele 1 > allele 2	Amino acid change	region	Allele frequency		Flanking sequence	rs ID
			Allele 1	Allele 2		
1753G>A	G36R	exon 2	1.000	0.000	TGAGAACGGC[G/A]GGGAGAAACC	
1910G>T		intron 2	0.473	0.527	TGTAACCCTA[G/T]TCATTCATTA	rs2070699
1918T>A		intron 2	0.999	0.001	TAGTCATTCA[T/A]TAGCGCTGGC	
2008G>A		intron 2	0.999	0.001	GTGCCTCAGT[G/A]GGGACAGTTT	
2107G>A		intron 2	0.999	0.001	TACTCATGAT[G/A]GGACAAGCAG	

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*28*). The nucleotide number was according to the reference sequences GenBank Accession ID: NT_007592.

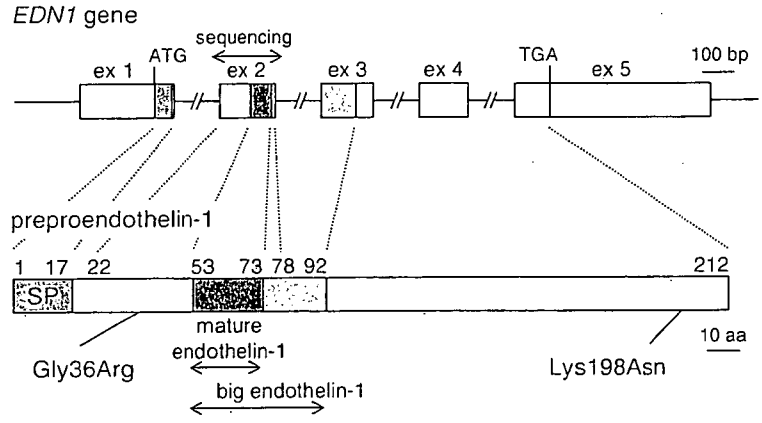

Fig. 1. *Genome and domain structure of human endothelin 1. Two missense mutations in endothelin-1, Gly36Arg (G36R) and Lys198Asn (K198N), are shown. The G36R mutation in preproendothelin-1 was identified in this study.*

significantly associated with DBP (CC+CT: 79.61±0.34 mmHg, n=842; TT: 84.13±2.06 mmHg, n=24; p=0.030).

The haplotypes composed of the 5 SNPs genotyped in this study are shown in Table 5. Seven inferred haplotypes with frequencies of more than 5% were examined to determine their association with hypertension in all patients and in two sub-populations (men and women). In women, the frequency of haplotype H5 in the hypertensive group was significantly lower than that in the normotensive group.

A Novel Missense Mutation in the preproEDN1 Polypeptide in Japanese Hypertensives

We sequenced the region of exon 2 of *EDN1* in 942 hypertensive patients with strong genetic background and secondary hypertension. The results are shown in Table 6. In this study, we were not able to detect any missense mutations within the mature EDN1 region. However, we identified one novel missense mutation, G36R, in *EDN1* in a heterozygous form in a male patient. The prevalence of this mutation was 0.05% in our Japanese hypertensive population. We tried to screen this missense mutation, G36R in *EDN1*, in our general population by the TaqMan-PCR method, but this genotyping failed due to technical problems.

Discussion

In this study, we used two different approaches to reveal the contribution of the EDN system to hypertension in two different populations, a general population and a hypertensive population, both from the Osaka region in Japan.

We genotyped 5 SNPs in *ECE1* and identified rs212528 as the hypertension/blood pressure susceptibility genetic variant. We used the currently available HapMap data from CHB-JPN to assess the coverage of haplotype blocks across the *ECE1* gene by 5 SNPs. The *ECE1* gene consisted of 6 haplotype blocks, in which rs212548 was present in block 2, two SNPs, rs212528 and rs212526, were present in block 3, and two SNPs, rs2038090 and rs2038089, were present in block 6, and the genotyped SNPs were estimated to cover approximately 90% of the haplotypes in block 2, 30% of those in block 3, and 90% of those in block 6, respectively. Two SNPs, rs212528 and rs212526, in block 3 had an r^2 of 0.031 and LOD score of 0.43, and rs2038090 and rs2038089 in block 6 had an r^2 of 0.163 and LOD score of 2.33.

In this study, the rs212528-T>C polymorphism in *ECE1* in

women was identified as the SNP conferring susceptibility for hypertension and blood pressure change. It is well known that the incidence of coronary artery disease shows a gender difference that may in part be related to the female sex hormones estrogen and progesterone. The literature provides evidence that estrogen inhibits EDN1 production (*26*). Furthermore, estrogen inhibits ECE-1 mRNA expression (*27*). These findings may explain the gender difference of *ECE1* polymorphisms for hypertension. The mean age of women in our population was 63.3 years. Despite the relatively advanced age of this population, we identified a contribution of the rs212528 polymorphism to hypertension and blood pressure change, while haplotypes containing the rs212528-C allele were not clearly associated with normotension or hypertension. The association might have been stronger if we had used a younger female population.

Another polymorphism, rs212526-C>T in intron 6, was associated with a blood pressure change in women and men. The mean DBP of the 996 women with the CC+CT genotype was 6.57 mmHg higher than that of the 18 women with the TT genotype ($p=0.003$), and the SBP change also showed the same trend—that is, women with the CC+CT genotype had higher blood pressure than women with the TT genotype ($p=0.044$) (Table 4). However, in men, the opposite trend was seen. The mean DBP of the 842 men with the CC+CT genotype was 4.52 mmHg lower than that of the 24 men with the TT genotype ($p=0.030$). Haplotype H5 containing the rs212528-T allele was significantly more prevalent in the normotensive group. This association also suggested that the T-allele of rs212528 was involved in blood pressure in women (Tables 3–5). Thus, the significance of rs212526 on blood pressure change should be evaluated using other population.

The association of SNP with hypertension and blood pressure change is at best marginally significant given the number of tests performed. All the p-values were more than 0.007. However, rs212528 is present in the *ECE1* gene, which encodes the endothelin-converting enzyme. In addition, this SNP showed a positive association with both hypertension and blood pressure change. Thus, we regarded this SNP as a hypertension candidate. SNP and blood pressure/hypertension described in the present study needs to be confirmed by another set of studies.

In the hypertensive population, we sequenced the coding region of the EDN1 polypeptide and its flanking region in 942 Japanese hypertensives and identified one novel missense mutation, G36R, that was not present in the EDN1 polypeptide but was present in the preproEDN-1 region (Fig. 1). At present, the effect of G36R mutation on the EDN1 function is not clear, because it was located far from the scissile site, the R52–C53 bond, by the furin-like enzyme. From the evolutionary point of view, G36 was conserved in humans, chimpanzees, cows, and dogs, but mice and rats have Val and chickens have Ala. The arginine residue at position 36 was not found in preproEDN1 in any species. To reveal the functional effect of this missense mutation on the processing of preproEDN1, an expression study of the mutant preproEDN1 is needed.

We have hypothesized that rare nonsynomymous mutations in candidate genes could collectively contribute to complex traits. In this model, the extensive sequence-based approaches focusing on identification of these mutations is necessary. So far, we have sequenced several hypertension candidate genes to evaluate whether rare variants could contribute to the etiology of hypertension. At present, however, whether rare variants contribute to hypertension is not clear due to the lack of *in vitro* or *in vivo* expression studies of the mutant protein (*14, 15, 17*). The exception was the nonsense mutation identified in the *RGS2* gene, which has been clearly shown to produce the defective protein (*16*). In this study, we identified one missense mutation, G36R, in preproEDN1. The further collection of such missense mutations in hypertension candidate genes could lead to an enhanced understanding of the etiology of essential hypertension.

In summary, we revealed that the rs212528 polymorphism in *ECE1* was associated with hypertension and blood pressure change. In earlier reports, the Lys198Asn polymorphism in *EDN1* showed a positive association with blood pressure elevation in overweight people (*3–5*). Thus, endothelin family gene polymorphisms might play an important role in the etiology of essential hypertension.

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We have found that high concentration of mercaptoethanol (for example 10 mM) or long incubation times (for example 30 minutes) causes a decrease in immunostaining intensity, possibly due to loss of epitopes through excessive reduction (data not shown). In our laboratory, incubation of a 2 mm-thick gel for 10 minutes in 1 mM β-mercaptoethanol has proven adequate (Fig. 1). These conditions are suitable for VWF concentrations from 6 IU/dl to 250 IU/dl (data not shown). β-mercaptoethanol is a toxic and highly pungent reagent, therefore appropriate precautions should be taken when using it.

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Age- and gender-related differences of plasma prothrombin activity levels

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Dear Sir,

Advancing age is an important risk factor for venous or arterial thrombosis in both sexes (1–3). Moreover, gender is associated with differences in the prothrombotic state and in the progression of atherosclerosis that occurs with aging (4, 5). Prothrombin is one of the dominant factors influencing thrombin generation (6), and the prothrombin G20210A mutation accompanied by an increased level of prothrombin poses a risk factor for venous or arterial thrombosis (7, 8). However, gender differences in age-related changes in plasma prothrombin activity have not been investigated until now. In the present study, we measured prothrombin activity in 742 individuals derived from a general Japanese population which was supposed to be free of prothrombin G20210A mutation (9).

The study population was composed of samples randomly selected from the residents of Suita, a city located in the second largest urban area in Japan (the Suita Study) (4). All subjects had been visiting the National Cardiovascular Center every two years since 1989 for regular health checkups. Only subjects who provided written informed consent to have a blood examination were enrolled in this study. We excluded subjects treated with oral anticoagulant therapy. Finally, 742 subjects, aged 36 to 85 years (mean age: 64 years), were included in this study. Spearman correlation analysis was used to assess the association between aging and the level of prothrombin activity within a given gender. For comparison between the two gender groups, the Mann-Whitney U test was used. Differences with a value of $p<0.01$ for the Spearman correlation analysis and $p<0.05$ for the Mann-Whitney U test were considered to be significant. Statistical calculations were performed using SPSS version 12.0 (SPSS Inc, Chicago, IL, USA). Prothrombin activity was measured according to a published method (10) with a modification. Briefly, 200 µl of 20 mM Tris-HCl, 0.14 M NaCl, pH 7.5 buffer containing 1 mg/ml of bovine serum albumin (TBSA) was added to 50 µl of plasma anticoagulated with 0.13% sodium citrate. Then, diluted plasma was incubated for 150 seconds at 37°C, and we detected ΔA/min at 405 nm after adding 50 µl of the reagent containing 6 mM $CaCl_2$, 0.5 mM Boc-Val-Pro-Arg-pNA as a thrombin substrate, 500 pM carinactivase-1 as a thrombin activator, and TBSA. Calibration was performed with a standard-human-plasma (Dade Behring GmbH, Marburg, Germany). The coefficient of intra-assay variation for prothrombin activity assay was 2.0%.

The mean ± SD of prothrombin activity level in men and women was 110.2 ± 17.0 (range: 54.5–158.5%) and 120.4 ± 17.4 (range: 57.5–194.4%), respectively. Figure 1 shows the age-related distribution (36–85 years) of prothrombin activity in 348 men (Fig. 1A) and 394 women (Fig. 1B). As a whole, a linear decrease of prothrombin activity level with age was observed in

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men (r=-0.34, p<0.0001), but not in women (r=-0.04, p=0.47). When prothrombin activity level was analyzed in 10-year age groups, significant decreases were observed in the men aged 46–55 years and 56–65 years (p<0.0001), aged 56–65 years and 76–85 years (p<0.05), and in the women aged 66–75 years and 76–85 years (p<0.0001). Levels of prothrombin activity were decreased in both sexes in the oldest age group (aged 76–85 years). With regards to gender-related change, the prothrombin activity level in the age group of 56–65 years, 66–75 years, and 76–85 years was significantly lower in men than in women.

In the present study, we showed the age-related decrease in the plasma prothrombin activity of men and gender-related change in the plasma prothrombin activity. These results contribute to the understanding of age-related hypercoagulability and to the practical institution of anticoagulant therapy in older patients. It has been established that thrombin generation increases with age in both sexes, evidenced by plasma prothrombin fragment F1+2 levels produced by the cleavage of prothrombin by factor Xa (11, 12). Age-related hypercoagulability does not likely stem from the prothrombin activity, because the prothrombin activity of men showed the age-related decrease, but it may result from some other mechanisms including decreased levels of anticoagulant proteins such as protein C and S (11, 13). We presented here the gender-related change of significantly lower prothrombin activity levels in men in the age of 56–85 years than in women. Men tend to develop thrombotic events including recurrent venous thrombosis (14), but this tendency was not related to the plasma level of prothrombin activity. Our work sheds further light on the point that, when considering relative hypercoagulability, gender-adjustment is necessary for the comparison of prothrombin activity levels.

With regards to anticoagulant therapy, the plasma levels of vitamin K-dependent coagulation factors decrease with increasing intensity of anticoagulation therapy (15). At the same time, the risks of major haemorrhage increase according to the intensity of anticoagulation therapy, especially in patients older than 80 years (16). Given our current study results, the markedly decreased prothrombin level in the age group of 76–85 years, especially in men, provides a potential mechanistic explanation for the increased rate of major haemorrhage observed in elderly patients receiving anticoagulant therapy.

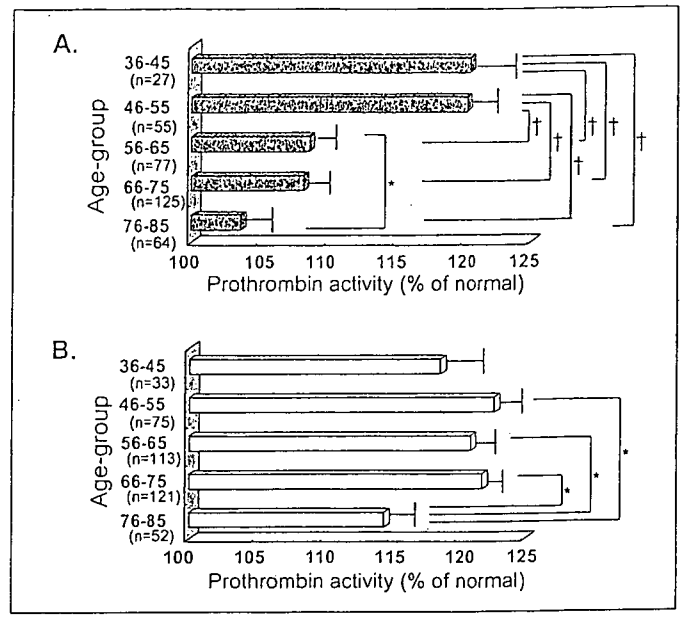

Figure 1: Age-related changes of plasma prothrombin activity levels according to gender (A: men, B: women). Populations aged from 36 to 85 years old were divided into five age groups by gender. Data are expressed as the mean ± SEM. *: P<0.05, †: P<0.0001, compared between two age groups of the same gender.

In conclusion, there are significant age- and gender-related differences in plasma prothrombin activity levels. In particular, the prothrombin activity level in men in the age group of 76–85 years was lower than that of any other age group in either gender.

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Sex Hormone and Gender Difference—Role of Testosterone on Male Predominance in Brugada Syndrome

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Testosterone in Brugada Syndrome. *Introduction:* **The clinical phenotype is 8 to 10 times more prevalent in males than in females in patients with Brugada syndrome. Brugada syndrome has been reported to be thinner than asymptomatic normal controls. We tested the hypothesis that higher testosterone level associated with lower visceral fat may relate to Brugada phenotype and male predominance.**

Methods and Results: **We measured body-mass index (BMI), body fat percentage (BF%), and several hormonal levels, including testosterone, in 48 Brugada males and compared with those in 96 age-matched control males. Brugada males had significantly higher testosterone (631 ± 176 vs 537 ± 158 ng/dL; $P = 0.002$), serum sodium, potassium, and chloride levels than those in control males by univariate analysis, and even after adjusting for age, exercise, stress, smoking, and medication of hypertension, diabetes, and hyperlipidemia, whereas there were no significant differences in other sex and thyroid hormonal levels. Brugada males had significantly lower BMI (22.1 ± 2.9 vs 24.6 ± 2.6 kg/m^2; $P < 0.001$) and BF% (19.6 ± 4.9 vs 23.1 ± 4.7%; $P < 0.001$) than control males. Testosterone level was inversely correlated with BMI and BF% in both groups, even after adjusting for the confounding variables. Conditional logistic regression models analysis showed significant positive and inverse association between Brugada syndrome and hypertestosteronemia (OR:3.11, 95%CI:1.22–7.93, $P = 0.017$) and BMI (OR:0.72, 95%CI:0.61–0.85, $P < 0.001$), respectively.**

Conclusions: **Higher testosterone level associated with lower visceral fat may have a significant role in the Brugada phenotype and male predominance in Brugada syndrome.** *(J Cardiovasc Electrophysiol, Vol. 18, pp. 415-421, April 2007)*

Brugada syndrome, gender, sex hormones, testosterone, body mass index

Introduction

Brugada syndrome is characterized by coved-type ST-segment elevation in the right precordial electrocardiographic (ECG) leads (V1–V3) and an episode of ventricular fibrillation (VF) in the absence of structural heart disease.[1-5] The prevalence of the disease is estimated to be up to 5 per 10,000 inhabitants and is one of the important causes of sudden cardiac death of middle-aged males, particularly in Asian countries including Japan.[4]

More than eight dozen distinct mutations in *SCN5A*, the gene encoding the α subunit of the sodium channel, have been so far identified in patients with Brugada syndrome and all mutations display an autosomal-dominant mode of transmission.[6,7] Therefore, males and females are expected to inherit the defective gene equally. However, more than 80% of patients in Western countries and more than 90% of patients in Asian countries affected with Brugada syndrome are males.[8] Recent experimental studies have unveiled the cellular mechanism of Brugada phenotype. The male predominance in the Brugada syndrome is suggested to be due, at least in part, to intrinsic differences in ventricular action potential (AP) between males and females.[9]

A male hormone, testosterone is reported to increase net outward currents[10-12] and is expected to accentuate Brugada phenotype, such as ST-segment elevation and subsequent episodes of VF in patients with Brugada syndrome. Testosterone is also known to decrease visceral fat.[13-15] Since patients with Brugada syndrome have been reported to be

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thinner than asymptomatic normal controls by Matsuo et al.,[16] we speculated that higher testosterone level associated with lower visceral fat may modulate Brugada phenotype and may relate to male predominance in patients with Brugada syndrome.

Methods

Patient Population and Data Collection

The study population consisted of 48 males with Brugada syndrome who agreed to participate in this study and showed Type 1 "coved" ST-segment elevation in V1–V3 leads[17] ranging in age from 30 to 69 years with a mean age of 50 ± 11 years (mean ± SD). Brugada males who were less than 30 years old and more than 70 years old were excluded from this study to minimize the influence of age on the basal sex hormonal levels including testosterone. Forty of the forty-eight Brugada males have been included in our previous clinical studies.[18-20] In all patients, physical examination, chest roentgenogram, laboratory values, echocardiography with wall motion analysis, and Doppler screening excluded structural heart diseases. The clinical, electrocardiographic, and electrophysiologic characteristics of the 48 Brugada males are shown in Table 1. Average age of the 48 Brugada males at diagnosis was 47 ± 12 years old. Aborted cardiac arrest or VF was documented in 21 males (44%), syncope alone in 11 males (23%), and 16 males (33%) were asymptomatic. Family history of sudden cardiac death (SCD) was observed in eight males (17%). An *SCN5A* coding region mutation was identified in seven (17%) of 42 males in whom genetic screening was conducted. Implantable cardioverter defibrillator (ICD) was implanted in all 32 symptomatic males with documented VF and/or syncope. ICD was also implanted in nine of 16 asymptomatic males due to induction of VF during the electrophysiologic study. Type 1 ST-segment elevation was recorded spontaneously in 43 males (90%) and was induced by sodium channel blockers in five males (10%). Complete right bundle branch block was observed in three males (6%). Late potential was recorded by a signal-average ECG system in 27 (59%) of 46 males. During the electrophysiologic study, VF requiring direct cardioversion for termination was induced in 32 (73%) of 44 males. Average HV interval was 46 ± 11 msec.

We first obtained data, such as the hormonal levels, visceral fat parameters, and ECG parameters in the 48 Brugada males prospectively between January and July in 2003, mainly at regular outpatient clinics for checking ICD. Only a Brugada male refused to participate during the recruitment of the case.

Thereafter, age-matched control males were randomly selected from the municipal population registry in Suita City. The hormonal and visceral fat data were collected sequentially between August and December in 2003. The municipal population registry in Suita City included 5,846 control subjects, among whom 1,052 males were age-matched to the 48 Brugada males. The 96 control males with a mean age of 50 ± 11 years were sequentially recruited from the age-matched 1,052 males. None of the recruited 96 control males refused to participate in this study. There were no significant differences in the clinical characteristics between the 96 control males and the remaining 956 age-matched males. Therefore, we had no way of knowing the body weight of the individuals who were selected to serve as controls from a very large database. Although K. Matsuo is a co-author of this study, none of the Brugada males and control males who appeared in the article by Matsuo[16] are included in the present study population.

All protocols were approved by the Ethical Review Committee in the National Cardiovascular Center. Written informed consent was obtained from all subjects.

Sex and Thyroid Hormonal Levels and Serum Electrolytes

Blood samples for analysis of basal hormone levels and serum electrolytes were obtained between 8:00 and 9:00 AM after an overnight fast. Plasma sex hormonal levels including testosterone, estradiol, DHEA-S, LH, and FSH were measured using commercially prepared immunoassay kits (testosterone, LH, and FSH: Chemiluminescent immunoassay [Bayer HealthCare, New York, NY, USA]; estradiol: Electrochemiluminescent immunoassay [Roche Diagnostics GmbH, Mannheim, Germany]; DHEA-S: Radioimmunoassay [Diagnostic Products Corporation, Los Angeles, CA, USA]). Thyroid hormonal levels including free T3, T4, and TSH, and serum electrolyte levels including sodium, potassium, and chloride were also measured.

Body Mass Index and Body Fat Percentage

Body weight (BW) was measured to the nearest 0.1 kg and height to the nearest cm. Body-mass index (BMI) was calculated as weight/height^2 (kg/m^2) as a parameter of visceral fat. We also measured body-fat percentage (BF%) by using body composition analyzer (Biospace Co., Ltd. Tokyo, Japan). These visceral fat parameters were measured just after blood sampling. In the 32 symptomatic Brugada males who had had documented VF and/or syncope, the BW and BMI were also measured within 48 hours after their clinical events during admission in our hospital or other emergent hospitals.

TABLE 1
Clinical, Electrocardiographic, and Electrophysiologic Characteristics in the 48 Brugada Males

Clinical characteristics	
Age at diagnosis (years)	47 ± 12
Aborted cardiac arrest or VF (%)	21/48 (44%)
Syncope alone (%)	11/48 (23%)
Asymptomatic (%)	16/48 (33%)
Family history of SCD	8/48 (17%)
SCN5A mutation	7/42 (17%)
ICD implantation	41/48 (85%)
Follow-up period (month)	41 ± 2
Arrhythmic event (%)	9/48 (19%)
Electrocardiographic characteristics	
Spontaneous coved-type ST elevation	43/48 (90%)
CRBBB (%)	3/48 (6%)
RR (msec)	939 ± 113
PQ interval (II) (msec)	186 ± 34
QRS duration (V2) (msec)	104 ± 18
Corrected QT interval (V5) (msec)	394 ± 27
ST amplitude at J point (V2) (mV)	0.32 ± 0.16
Late potential (%)	27/46 (59%)
Electrophysiologic characteristics	
Induction of VF	32/44 (73%)
Mode (Triple/Double/Single)	16/15/1
HV interval (msec)	46 ± 11

CRBBB = complete right bundle branch block; ICD = implantable cardioverter defibrillator; SCD = sudden cardiac death; VF = ventricular fibrillation.

ECG Parameters

In the 48 males with Brugada syndrome, 12-lead ECG was recorded just before blood sampling, and ECG parameters were assessed by an investigator (WS) blinded to clinical information. The ECG parameters included RR interval, PQ interval measured in lead II, QRS interval measured in lead V2, QT interval, corrected QT (QTc) interval measured in leads V5, and ST amplitude at J point measured in lead V2.

Statistical Analysis

We first conducted univariate analysis by using unpaired *t*-test to compare each data between the Brugada males and the control males. Since several confounding variables, such as age, exercise (none, sometimes, regularly), stress (none, sometimes, regularly), current smoking (no, yes), and medication (no, yes) of hypertension, diabetes, and hyperlipidemia may affect the hormonal levels including testosterone level and the visceral fat parameters, analysis of covariance (ANCOVA) was used to compare least square mean values between the Brugada males and the control males adjusting for these confounding variables. Pearson's correlation coefficients were calculated between the testosterone level and the visceral fat parameters. Partial correlation coefficients were calculated between the testosterone level and the visceral fat parameters after adjusting for age, exercise, stress, current smoking, and medication. Moreover, conditional logistic regression models were used to calculate odds ratios and 95% confidence intervals adjusting for age, BMI, exercise, stress, current smoking, hypertension, diabetes, and hyperlipidemia. Hypertestosteronemia was defined as serum testosterone levels $\geq$700 ng/dL, which is 75 percentiles of testosterone levels among case and control combined groups. In the 32 Brugada males with documented VF and/or syncope, a paired *t*-test was used to compare the visceral fat parameters at the clinical cardiac events and at the measurement of hormonal and visceral fat data. A two-sided P value below 0.05 was considered to indicate significance. All statistical analyses were performed by using SAS software, Ver 8.2.

Results

Hormonal Levels, Serum Electrolytes, and Visceral Fat

Table 2 illustrates univariate analysis for comparing sex and thyroid hormonal levels, serum electrolytes, and visceral fat parameters between the two groups. Testosterone level was significantly higher in the Brugada males than in the control males, whereas there were no significant differences in other sex hormonal levels; estradiol, DHEA-S, LH, FSH, and thyroid hormonal levels; T3, T4, and TSH. Serum sodium, potassium, and chloride levels were all significantly higher in the Brugada males than in the control males. BMI, BF%, and BW were all significantly lower in the Brugada males than in the control males. All variables followed normal distribution, both in the 48 Brugada and 96 control males.

The comparison of the confounding variables that may affect the hormonal levels and the visceral fat parameters between the 48 Brugada males and the 96 control males was shown in Table 3. Even after adjusting for age, exercise, stress, current smoking, and medication (hypertension, diabetes, and hyperlipidemia), the testosterone level, serum sodium, potassium, and chloride levels were all significantly higher, and the visceral fat parameters were significantly lower in the 48 Brugada males than in the 96 control males (Table 4). There were also significant differences in these parameters between the 24 definite Brugada males with documented VF and/or *SCN5A* mutations and the 96 control males after adjusting for the confounding variables (Table 4).

Correlation between Testosterone, Visceral Fat, and Serum Electrolytes

Testosterone level was inversely correlated with all visceral fat parameters, BMI, BF%, or BW in both the Brugada males and the control males, even after adjusting for age,

TABLE 2

Sex and Thyroid Hormonal Levels, Serum Electrolytes, and Visceral Fat Parameters in the 48 Brugada Males and the 96 Age-Matched Control Males

	Brugada Males (n = 48)	Control Males (n = 96)	P Value
Sex hormones			
Testosterone (ng/dL)	631 ± 176	537 ± 158	0.002
Estradiol (pg/mL)	28.9 ± 7.6	31.1 ± 12.6	0.263
DHEA-S (ng/mL)	1,901 ± 850	1,966 ± 861	0.668
LH (mIU/mL)	4.6 ± 2.6	3.9 ± 2.0	0.073
FSH (mIU/mL)	6.2 ± 4.9	5.0 ± 2.9	0.066
Thyroid hormones			
Free T3 (pg/mL)	3.3 ± 0.4	3.4 ± 0.3	0.360
Free T4 (ng/dL)	1.3 ± 0.1	1.3 ± 0.2	0.089
TSH (μIU/mL)	1.9 ± 1.4	1.7 ± 1.4	0.619
Serum electrolytes			
Sodium (mEq/L)	143.7 ± 2.0	142.6 ± 2.0	0.003
Potassium (mEq/L)	4.6 ± 0.3	4.3 ± 0.3	<0.001
Chloride (mEq/L)	105.1 ± 2.1	103.6 ± 2.1	<0.001
Viceral fat			
BMI (kg/m^2)	22.1 ± 2.9	24.6 ± 2.6	<0.001
BF% (%)	19.6 ± 4.9	23.1 ± 4.7	<0.001
BW (kg)	62.9 ± 9.7	70.0 ± 8.6	<0.001

Values are mean ± SD where indicated.
BMI = body-mass index; BF% = body-fat percentage; BW = body weight.

TABLE 3

Comparison of the Confounding Variables Between the 48 Brugada Males and the 96 Age-Matched Control Males

	Brugada Males (n = 48)	Control Males (n = 96)	P Value
Exercise			
None (%)	39.6	44.8	
Sometimes (%)	41.6	43.8	
Regularly (%)	18.8	11.5	0.482
Stress			
None (%)	27.1	21.9	
Sometimes (%)	54.2	54.2	
Regularly (%)	18.8	24.0	0.684
Current smoking (%)	25.0	27.1	0.789
Medication			
Hypertension (%)	20.8	19.8	0.883
Diabetes (%)	2.1	13.5	0.028
Hyperlipidemia (%)	10.4	5.2	0.246

TABLE 4
Testosterone, Serum Electrolytes, and Visceral Fat Parameters in the Brugada Males and the 96 Age-Matched Control Males after Adjusting for Confounding Variables

	Brugada Males	Control Males (n = 96)	P Value
ALL Case (n = 48)			
Testosterone (ng/dL)	631 ± 44	538 ± 40	0.003
Sodium (mEq/L)	144.2 ± 0.5	143.2 ± 0.5	0.007
Potassium (mEq/L)	4.6 ± 0.1	4.3 ± 0.1	<0.001
Chloride (mEq/L)	105.5 ± 0.5	103.9 ± 0.5	<0.001
BMI (kg/m^2)	22.3 ± 0.7	24.9 ± 0.7	<0.001
BF% (%)	20.0 ± 1.3	23.9 ± 1.1	<0.001
BW (kg)	63.4 ± 2.4	70.1 ± 2.1	0.001
Definite Brugada case with VF and/or *SCN5A* (n = 24)			
Testosterone (ng/dL)	656 ± 59	550 ± 48	0.009
Sodium (mEq/L)	143.9 ± 0.7	142.9 ± 0.6	0.042
Potassium (mEq/L)	4.7 ± 0.1	4.4 ± 0.1	<0.001
Chloride (mEq/L)	105.2 ± 0.7	103.9 ± 0.6	0.006
BMI (kg/m^2)	21.5 ± 1.0	24.5 ± 0.8	<0.001
BF% (%)	19.9 ± 1.7	24.1 ± 1.4	<0.001
BW (kg)	60.5 ± 3.1	69.2 ± 2.5	0.001

Values are mean ± SE adjusted for age, exercise, stress, current smoking, and medication of hypertension, diabetes and hyperlipidemia. BMI = body-mass index; BF% = body-fat percentage; BW = body weight; VF = ventricular fibrillation.

exercise, stress, current smoking, and medication (Brugada: BMI, r = −0.394, P = 0.011; BF%, r = −0.390, P = 0.012; BW, r = −0.335, P = 0.032; Control: BMI, r = −0.333, p = 0.002; BF%, r = −0.333, P = 0.001; BW, r = −0.305, P = 0.004), suggesting that Brugada males had higher testosterone level associated with lower visceral fat compared with control males (Fig. 1). No significant correlations were observed between other serum electrolytes and testosterone level or visceral fat parameters. Testosterone level was not correlated with age, even after adjusting for exercise, stress, current smoking, and medication (r = 0.007, P = 0.947).

Conditional Logistic Regression Models Analysis

Conditional logistic regression models analysis showed significant positive and inverse association between Brugada syndrome, hypertestosteronemia (Odd Ratio (OR): 3.11, 95%CI: 1.22–7.93, P = 0.017), and BMI (OR: 0.72, 95%CI: 0.61–0.85, P < 0.001), respectively (Table 5). Other variables did not significantly increase or decrease risks of Brugada syndrome (Table 5).

Visceral Fat at Clinical Cardiac Events in Brugada Males

In the 32 symptomatic Brugada males with documented VF and/or syncope, the time-span between the clinical cardiac events and the measurement of hormonal and the visceral fat data was 42 ± 32 months (mean ± SD, 1–99 months). The BMI and BW at the clinical cardiac events (VF or syncope) were significantly lower than those at the measurement of hormonal and visceral fat data (BMI, 21.0 ± 2.6 vs 22.1 ± 2.9 kg/m^2; BW, 60.0 ± 8.9 vs 62.9 ± 9.7 kg: P < 0.001, respectively).

Testosterone versus ECG Parameters, Symptoms or SCN5A Mutation in Brugada Males

Baseline electrocardiographic data of the 48 Brugada males are shown in Table 1. No significant correlations were observed between testosterone level and ECG parameters, including ST amplitude (r = −0.123, P = 0.406) and QTc interval (r = −0206, P = 0.160), in the 48 Brugada males. There was no significant difference in testosterone level between 32 symptomatic and 16 asymptomatic Brugada males (649 ± 185 vs 593 ± 157 ng/dL: P = 0.298). No significant difference was observed in testosterone level between 43 Brugada males with spontaneous Type 1 ST-segment elevation and five Brugada males with sodium channel blocker-induced Type 1 ST-segment elevation (624 ± 171 vs 688 ± 230 ng/dL: P = 0.448). Testosterone level was also no different between seven Brugada males with *SCN5A* mutation and 41 Brugada males without *SCN5A* mutation (700 ± 198 vs 619 ± 172 ng/dL: P = 0.261).

Follow-Up

Arrhythmic events occurred in nine (19%) of 48 Brugada males during average follow-up periods of 41 ± 2 months after blood sampling for the present study (Table 1). In more detail, arrhythmic events appeared in eight (38%) of 21 Brugada males with a history of aborted cardiac arrest or VF, in one (9%) of 11 Brugada males with syncope alone, but did not appear in any (0%) of 16 asymptomatic Brugada males.

Discussion

The major findings of the present study were: (1) Brugada males had significantly higher testosterone level, serum sodium, potassium, and chloride level, and significantly lower BMI, BF%, and BW than those in control males by univariate analysis, even after adjusting for age, exercise, stress, current smoking, and medications related to hypertension, diabetes and hyperlipidemia. (2) Testosterone level was inversely correlated with the BMI, BF%, and BW in both Brugada males and control males, even after adjusting for the confounding variables. (3) Conditional logistic regression models analysis showed strong positive association between Brugada syndrome and higher testosterone level (hypertestosteronemia) and strong inverse association between Brugada syndrome and BMI.

Testosterone in Brugada Phenotype and Male Predominance

For the past decade, numerous clinical, experimental, and molecular genetic studies have elucidated Brugada syndrome as a distinct clinical entity.[1-5,17] However, several problems remain unresolved, such as genetic heterogeneity, ethnic difference, and gender difference.[7] Di Diego and Antzelevitch recently suggested the cellular basis for male predominance in Brugada syndrome by using arterially perfused canine right ventricular wedge preparations.[9] Transient outward current (I_{to})-mediated phase 1 AP notch was larger in male dogs than in female dogs in the right ventricular epicardium, but not in the left ventricular epicardium, responsible for the male predominance in the Brugada phenotype. Recent clinical studies suggested that male hormone testosterone might be attributable to gender difference of the prevalence in this

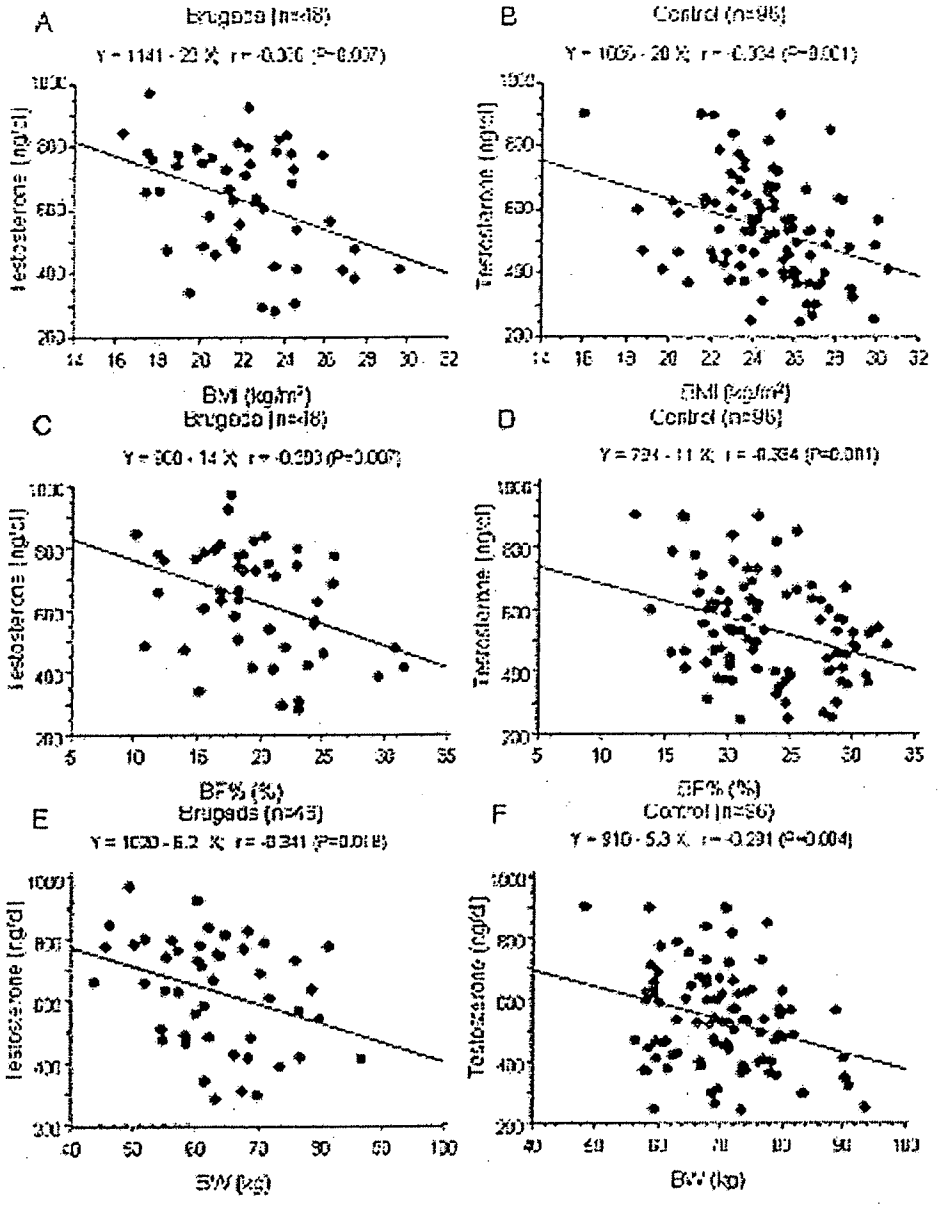

Figure 1. *Correlation between testosterone level and visceral fat parameters; body mass index (BMI) (A and B), body fat percentage (BF%) (C and D), and body weight (BW) (E and F) in the 48 Brugada males and the 96 age-matched control males. Testosterone level was inversely correlated with the BMI, BF%, or BW in both Brugada males and control males.*

syndrome. Matsuo et al. reported two cases of asymptomatic Brugada syndrome in whom typical coved ST-segment elevation disappeared following orchiectomy as therapy for prostate cancer,[21] indicating that testosterone may contribute to the Brugada phenotype in these two cases. Several experimental studies reported that testosterone increased outward potassium currents, such as the rapidly activating component (I_{Kr})[10,11] and the slowly activating component (I_{Ks})[12] of the delayed rectifier potassium current, and the inward rectifier potassium current (I_{K1}),[11] or decreased inward L-type calcium current (I_{Ca-L}).[12] Since the maintenance of the AP dome is determined by the fine balance of currents active at the end of phase 1 of the AP (principally I_{to} and I_{Ca-L}),[22,23] any agents that increase outward currents or decrease inward currents can increase the magnitude of the AP notch, leading to loss of the AP dome (all-or-none repolarization) in the epicardium, but not in the endocardium, contributing to a significant voltage gradient across the ventricular wall during ventricular activation, thus augmenting ST-segment elevation, the Brugada phenotype.[24] Therefore, testosterone would be expected to accentuate the Brugada phenotype. In the present study, males with Brugada syndrome had significantly higher testosterone level than age-matched control males, even after adjusting for age, exercise, stress, current smoking, and medication (hypertension, diabetes, and hyperlipidemia), which may affect the testosterone level. Moreover, conditional logistic regression models analysis showed strong positive association between Brugada syndrome and higher testosterone level (OR: 3.11). Our data suggest a significant role of testosterone, male hormone, in the Brugada phenotype. The

TABLE 5
Odds Ratios of Presence of Hypertestosteronemia and Confounding Risk Factors for Brugada Syndrome in Males

Variable	Odd Ratio	95% Confidence Interval	P Value
Hypertestosteronemia	3.11	1.22–7.93	0.017
Age	0.99	0.95–1.03	0.637
BMI	0.72	0.61–0.85	<0.001
Exercise	1.57	0.87–2.83	0.135
Stress	0.69	0.35–1.35	0.277
Current smoking	0.71	0.26–1.90	0.493
Hypertension	3.12	0.85–11.45	0.087
Diabetes	0.13	0.01–1.27	0.079
Hyperlipidemia	2.14	0.44–10.49	0.348

Hypertestosteronemia was defined as serum testosterone levels ≥ 700 ng/dL.

data also indicate that the male predominance in the Brugada phenotype is at least in part due to testosterone, which is present only in males.

Lower Visceral Fat May Be a Predictor for Brugada Phenotype

Matsuo et al. recently reported in their epidemiologic study that cases with the Brugada-type ECG had significantly lower BMI than that in control subjects.[16] Similarly, in the present study, males with Brugada syndrome had significantly lower visceral fat parameters, BMI, BF%, and BW than those in age-matched control males, even after adjusting for several confounding variables. Moreover, conditional logistic regression models analysis showed strong inverse association between Brugada syndrome and BMI (OR: 0.72). All of the visceral fat parameters were inversely correlated with testosterone level in both Brugada and control males, even after adjusting for the confounding variables. It has been well demonstrated that testosterone level in obese males is decreased compared to normal males of similar age.[13] Tsai et al. reported that lower baseline total testosterone level independently predicted an increase in visceral fat in the Japanese-American male cohort for 7.5 years.[15] Reversely, Marin et al. reported that testosterone treatment of middle-aged abdominally obese males was followed by a decrease of visceral fat mass measured by computerized tomography.[14] These data suggest that primarily higher level of testosterone in Brugada males compared to that in control males may result in lower visceral fat in Brugada males, which would be an "innocent bystander" sign of Brugada phenotype. In reverse, if primary lower visceral fat (body weight loss) would result in higher testosterone level, the weight loss could be a trigger for Brugada phenotype, just like fever is.[25] It is noteworthy that the visceral fat parameters at the clinical cardiac events (VF or syncope) in the 32 symptomatic Brugada males were significantly lower than those at the time of blood sampling for this study. This indicates that testosterone level is expected to be additively higher at the clinical cardiac events, which may contribute to spontaneous episodes of VF or syncope.

Other Hormonal Levels and Serum Electrolytes

Estradiol, female hormone, is reported to reduce the expression of Kv4.3 channels, which are important molecular components of I_{to} currents.[26] However, in contrast to testosterone, other sex hormonal levels including estradiol were not different between the Brugada males and the control males in the present study. Although thyroid hormones are also demonstrated to alter membrane currents, such as I_{to} and I_{Ca-L},[27,28] no significant differences were observed in the thyroid hormonal levels between the two groups in the present study.

On the other hand, serum sodium, potassium, and chloride levels were all significantly higher in the Brugada males than in the control males, even after adjusting for several confounding variables. Recently, many agents and conditions that cause an outward shift in current activity at the end of phase 1 AP have been known to unmask ST-segment elevation, as found in the Brugada syndrome, leading to the acquired form of this disorder.[4,29] Electrolyte abnormalities, such as hyperkalemia, are reported to amplify ST-segment elevation like that in Brugada syndrome.[30] The lower visceral fat found in the Brugada males is expected to decrease serum level of insulin, leptine, a novel adipocyte-derived hormone, or ghrelin, a novel growth hormone-releasing peptide, suppressing β-adrenergic receptor or plasma norepinephrine level, resulting in an increase of serum potassium level.[31,32] Further studies including measurement of levels of insulin, leptine, and ghrelin will be required to elucidate the precise mechanism.

Study Limitations

Although the testosterone level was significantly higher in the Brugada males than in the control males, no statistically significant correlations were observed between the testosterone level and the ST amplitude in the Brugada males. The degree of the ST-segment elevation is variable between Brugada patients because it is influenced by several factors other than sex hormonal levels or electrolytes levels, such as basal autonomic tone, presence of *SCN5A* mutation, or probably intrinsic current density of I_{to}, etc., in the right ventricular epicardial cells. The threshold of ST-segment elevation for spontaneous induction of VF also varies between Brugada patients. Therefore, the Brugada phenotype, such as ST-segment elevation or spontaneous induction of VF, may correlate with the testosterone level day to day individually (intra-personally) in each Brugada male, but may not correlate among the pooled data obtained from many Brugada males, probably due to inter-person difference of the ST-segment elevation.

There were no significant differences in testosterone level between symptomatic and asymptomatic Brugada males, between Brugada males with spontaneous ST elevation and those with sodium channel blocker-induced ST elevation, or between Brugada males with and without *SCN5A* mutation, all of which are probably due to a relatively small number of Brugada males in the present study. Further evaluation with increasing number of Brugada males will be required.

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Circ J 2007; 71: 191–195

Characterization of Subclinical Thyroid Dysfunction From Cardiovascular and Metabolic Viewpoints

— The Suita Study —

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Background Subclinical hypothyroidism, defined as high serum thyroid-stimulating hormone (TSH) levels and normal serum free-triiodothyronine (fT3) and serum free-thyroxine (fT4) levels, is a common medical problem among the elderly, but it is unclear whether it should be treated with thyroid hormone replacement therapy.
Methods and Results A cross-sectional study of 3,607 participants in a community health survey in Suita, in the northern part of Osaka, was performed. Participants were categorized into 5 groups: normal, hyperthyroidism, hypothyroidism, subclinical hypothyroidism, and subclinical hyperthyroidism. The association between each group and various phenotypes was examined, in relation to cardiovascular disease and metabolic syndromes. Serum TSH levels increased and fT3 and fT4 levels decreased with age. A total of 14.6% of subjects aged 70–80 years and 20.1% of subjects aged older than 80 years were classified as having subclinical hypothyroidism. Subclinical hypothyroidism was not associated with glycol-hemoglobin A1c, body mass index, pulse rate, hypertension, total cholesterol, high-density lipoprotein cholesterol or triglyceride levels or intima–media thickness. It was only associated with higher fasting blood glucose and glycol-hemoglobin A1c levels compared with euthyroidism.
Conclusions The present observation does not support the need for treatment of subclinical hypothyroidism or subclinical hyperthyroidism. (*Circ J* 2007; 71: 191–195)

Key Words: Blood sugar; Diabetes; Lipids; Subclinical hyperthyroidism; Subclinical hypothyroidism

Subclinical hypothyroidism (SCH), defined as high serum thyroid-stimulating hormone (TSH) levels and normal levels of serum free-triiodothyronine (fT3) and serum free-thyroxine (fT4), is a common medical problem among the elderly. The prevalence of SCH has been reported to be 4–10% in the general population and up to 20% in women older than 60 years![1–3] The incidence of SCH is 2.1–3.8% per year in thyroid-antibody-positive subjects and 0.3% per year in thyroid-antibody-negative subjects[4]

Serum lipid levels in SCH have been reported as either normal[5] or elevated[6,7] In the Tromsø study, low-density lipoprotein-cholesterol (LDL-C) levels were significantly higher in subjects with SCH compared with controls[7] and, moreover, they were reduced with thyroxine treatment. In addition, associations between left ventricular function and SCH have been widely investigated, but the findings are controversial. Some studies have shown an association between SCH and poor left ventricular function and others have not[8] Moreover, the positive association between arterial stiffness and hypothyroidism, even in the subclinical stage, has been reported[9,10] Subclinical hyperthyroidism has been associated with a higher prevalence of atrial fibrillation (AF) and increased heart rate[8] but not with elevated serum lipid levels[6]

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In the present study we investigated whether subclinical thyroid dysfunction in Japanese individuals is associated with various phenotypes related to cardiovascular disease and metabolic syndromes.

Methods

Study Population

The selection criteria and design of the Suita study have been described previously![11–13] Serum TSH, fT3, and fT4 levels were measured in 3,607 subjects who were not being treated for thyroid disease. The present study was approved by the Ethics Committee of the National Cardiovascular Center, and all subjects provided written informed consent. We categorized patients into 5 groups: normal (normal levels of serum TSH [0.436–3.78 μU/ml], fT3 [2.1–4.1 pg/ml] and fT4 [1.0–1.7 ng/dl]), hyperthyroidism (low levels of TSH and high levels of fT3 and/or fT4), hypothyroidism (high levels of TSH and low levels of fT3 and/or fT4), SCH (high levels of TSH and normal levels of fT3 and fT4), and subclinical hyperthyroidism (low levels of TSH and normal levels of fT3 and fT4)![14] Body mass index (BMI) was calculated as body weight (kg) divided by height in square meters.

The intima–media thickness (IMT) was measured on longitudinal scan of the common carotid artery at a point 10 mm proximal from the beginning of the dilation of the bulb![11]

Serum TSH, fT3, and fT4 Levels

Fasting serum samples were collected at study entry and stored at −80°C until tests were run. Serum TSH was mea-

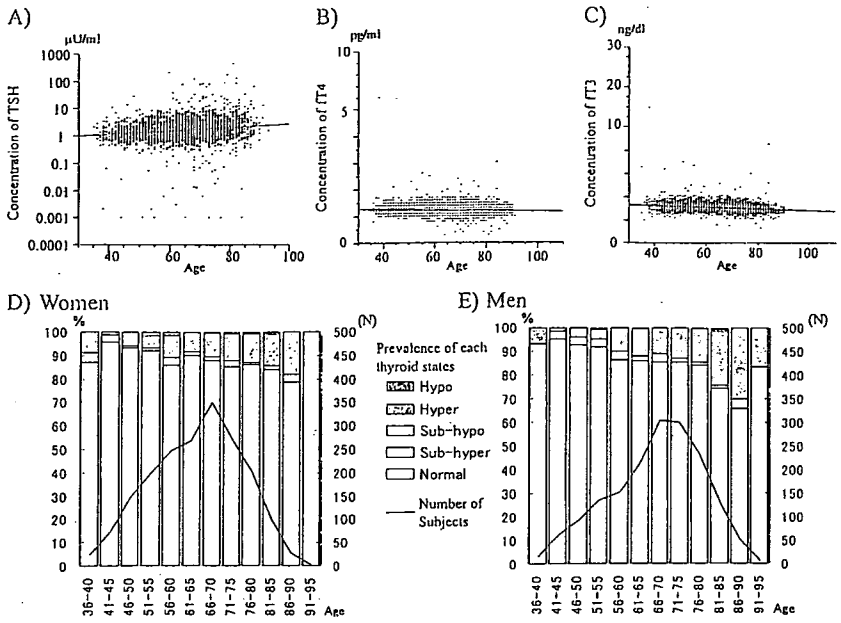

Fig 1. Serum TSH, fT3, and fT4 hormone levels and age. (A–C) Correlation between serum TSH (A), fT3 (B), and fT4 levels (C) and age are plotted (grey dot). Serum TSH levels increased with age. There was a linear correlation between log transferred serum TSH levels and age. Serum fT3 and fT4 levels decreased with age. There was a linear correlation between these thyroid hormone levels and age ($p<0.01$). (D–E) The prevalence of women (D) and men (E) in each thyroid state according to age is shown. The total number of subjects according to age is shown by the black line. The prevalence of subclinical hypothyroid in both men and women increased with age. However the prevalence of subclinical hyperthyroidism did not increase with age. TSH thyroid-stimulating hormone; fT3, serum free-triiodothyronine; fT4 serum free-thyroxine.

sured by a chemiluminescent immunoassay kit (Mitsubishi Kagaku BCL, Abbott Laboratories, Chicago, IL, USA), as were serum fT3 and fT4 levels (Mitsubishi Kagaku BCL, Bayer, Leverkusen, Germany).

Statistical Analysis

Values are expressed as mean±standard deviation. All statistical analyses were performed with the JMP statistical package (SAS Institute Inc, Cary, NC, USA). One-way analysis of variance tests were used to determine whether an association existed among thyroid status and BMI (adjusted for age and sex), systolic and diastolic blood pressures (SBP: adjusted for age, BMI, and sex; DBP: adjusted for age, BMI, and sex), pulse rate (PR; adjusted for age and sex), glycol-hemoglobin A1c (HbA1c: adjusted for age, BMI, and sex), fasting blood glucose levels (FBG: adjusted for age, BMI, and sex), high-density lipoprotein (HDL: adjusted for cholesterol BMI, sex, age, number of cigarettes/day, and alcohol consumption [g/day]), total cholesterol (TC: adjusted for age, BMI, and sex), triglycerides (TG: adjusted for age, BMI, and sex) and IMT. Simple correlation analyses were used to determine whether an association existed between normal states and each thyroid state, as well as between the variables assessed after adjusting for confounding factors. Logistic analysis was used to determine whether an association existed among thyroid status and the prevalence of AF.

Results

Serum concentrations of TSH increased and serum concentrations of fT3 and fT4 decreased with age (Figs 1A–C). Similarly, the number of men and women with SCH also increased with age (Figs 1D,E). Overall, 11.6% of women aged 71–80 years and 13.8% of women aged older than 80 years were classified into the SCH category (Fig 1D). For men, 13.4% aged 71–80 years and 24.5% older than 80 years were classified into the SCH category (Fig 1E).

The characteristics of the study population are shown in Table 1. Participants included 3,130 normal subjects, 19 with hyperthyroidism, 4 with hypothyroidism, 77 with subclinical hyperthyroidism, and 377 with SCH. The prevalence of SCH was 10.45% and that of subclinical hyperthyroidism was 2.13%. Compared with normal individuals, subjects with SCH were significantly older (69.02±10.43 years vs 64.22±11.29 years, respectively; $p<0.05$) and had significantly lower serum fT3 levels (2.99±0.33 ng/dl vs 3.12±0.32 ng/dl, respectively; $p<0.01$, adjusted for age and sex) and fT4 levels (1.09±0.19 pg/ml vs 1.24±0.15 pg/ml, respectively; $p<0.01$, adjusted for age and sex). On the other hand, when individuals with subclinical hyperthyroidism were compared with normal subjects, they showed significantly higher concentrations of fT4 (1.34±0.18 pg/ml vs 1.24±0.15 pg/ml, respectively; $p<0.01$, adjusted for age and sex). Subjects with overt hyperthyroidism had significantly lower HDL ($p<0.01$) and TC levels ($p<0.01$) and higher PR ($p<0.01$) than subjects with normal thyroid function. Subjects with overt hypothyroidism had significantly higher TC levels ($p<0.01$) than subjects with normal thyroid function. Characteristics associated with hyper- and hypothyroid subjects were as reported previously.[15]

Characteristics of subjects with normal and subclinical thyroid dysfunction are shown in Table 2. The PR among subjects not being treated for arrhythmia was not significantly associated with subclinical hyperthyroidism or SCH after adjusting for age and sex. Blood pressure among subjects not being treated for hypertension was not significantly associated with subclinical hyperthyroidism or SCH after adjusting for appropriate confounding factors. Among subjects not being treated for hypertriglyceridemia, TG levels were not significantly associated with subclinical hyperthyroidism or SCH compared with normal subjects. HDL-cholesterol and TC levels were not significantly associated with subclinical hyperthyroidism or SCH subjects not being treated for hyperlipidemia. Among all subjects not receiving treatment for diabetes, FBG was significantly associated with SCH ($p<0.01$). Subjects with subclinical hyperthyroidism had significantly higher HbA1c ($p<0.05$) and FBG ($p<0.01$) levels than normal subjects. The numbers of subjects not being treated for each disease is shown in Table 2.

The prevalence of AF in subjects with subclinical hyper-

Table 1 Characteristics of the Study Population

	Normal thyroid	Hyperthyroidism	Hypothyroidism	Subclinical hyperthyroidism	Subclinical hypothyroidism	Total
N (%)	3,130 (86.78)	19 (0.53)	4 (0.11)	77 (2.13)	377 (10.45)	3,607 (100)
Male (%)	1,442 (46.07)	5 (26.32)	3 (75.0)	39 (50.65)	203 (53.85)	1,692 (44.55)
Age (years)	64.22±11.29	62.16±12.85	76.50±8.43	63.94±10.85	69.02±10.43‡	64.70±11.29
BMI	22.82±3.11	22.73±3.48	24.02±0.58	22.77±3.03	22.84±3.34	22.82±3.14
SBP (mmHg)	129.24±19.65	131.32±20.63	135.50±10.47	125.57±15.45	131.93±19.06	129.41±19.69
DBP (mmHg)	77.82±10.09	76.11±11.95	71.50±15.37	75.03±10.76	77.97±9.61	77.74±10.17
PR (beats/min)§	66.10±8.25	72.42±9.74‡	62.00±3.27	67.12±9.38	66.51±8.35	66.20±8.38
FBG (mg/dl)¶	99.75±21.32	105.16±40.89	99.00±4.97	106.79±38.83†	98.64±18.68	99.79±21.67
HbA1c (%)¶	5.49±0.74	5.47±0.86	5.57±0.31	5.73±1.49†	5.54±0.77	5.50±0.77
HDL-C (mg/dl)	60.56±15.52	51.11±15.81‡	55.25±16.80	58.71±17.01	58.47±16.01	60.20±15.61
TC (mg/dl)	208.26±32.09	185.58±35.83‡	247.25±55.05†	206.79±33.26	206.34±35.48	207.99±32.53
TG (mg/dl)	106.90±74.77	111.74±64.30	156.00±61.36	123.12±72.03	113.30±69.72	108.16±74.59
Prevalence of MI (%)	40 (1.28)	0 (0.00)	0 (0.00)	2 (2.60)	7 (1.86)	49 (1.36)
Prevalence of CVA (%)	89 (2.84)	0 (0.00)	0 (0.00)	3 (3.90)	13 (3.45)	105 (2.91)
Prevalence of AF (%)	44 (1.41)	0 (0.00)	0 (0.00)	2 (2.60)	15 (3.98)	61 (1.69)
TSH (μU/ml)	1.723±0.824	0.10±0.20	209.85±171.57‡	0.27±0.13*	8.32±11.42‡	2.60±9.51
fT4 (ng/dl)	1.24±0.15	2.26±0.97‡	0.35±0.0‡	1.34±0.18‡	1.09±0.19‡	1.23±0.20
fT3 (pg/ml)	3.12±0.32	6.75±5.77‡	1.65±0.29‡	3.18±0.46	2.99±0.33‡	3.12±0.59

†$p<0.05$ and ‡$p<0.01$.
P values are for comparisons with normal thyroid subjects after adjustment for appropriate confounding factors.
Values are mean±standard deviation.
§*Number of patients treated for arrhythmia was 61 in normal subjects, 1 in subclinical hyperthyroidism subjects, and 9 in subclinical hypothyroidism subjects;* ¶*number of patients treated for diabetes was 150 in normal subjects, 1 in hyperthyroidism subjects, 7 in subclinical hyperthyroidism subjects, and 22 in subclinical hypothyroidism subjects.*
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; FBG, fasting blood glucose levels; Hb, hemoglobin; HDL, high-density lipoprotein; C, cholesterol; TC, total cholesterol; TG, triglyceride; MI, myocardial infraction; CVA, cerebrovascular accident; AF, atrial fibrillation; TSH, thyroid-stimulating hormone; fT4, free-thyroxine; fT3, free-triiodothyronine.

Table 2 Characteristics of the Subjects in the Normal and Subclinical Thyroidism Groups

	Normal thyroid	Subclinical hyperthyroidism	Subclinical hypothyroidism	ANOVA p-value**
N	3,130	77	377	
BMI	22.82±3.11	22.77±3.03	22.84±3.34	NS
N‡	2,355	57	270	
SBP (mmHg)	124.95±18.37	122.53±20.00	127.74±17.98	NS
DBP (mmHg)	76.67±9.98	73.49±10.99	76.93±9.50	NS
N§	3,057	75	364	
PR (beats/min)	66.12±8.23	67.33±9.40	66.47±8.27	NS
N¶	2,976	70	355	
HbA1c	5.40±0.56	5.56±1.34*	5.41±0.48	<0.05
FBG (mg/dl)	97.07±15.27	103.6±36.73†	95.58±11.57†	<0.0001
N††	3,084	73	371	
TG (mg/dl)	105.95±74.12	121.52±75.98	112.73±69.70	NS
N‖	2,726	63	320	
HDL (mg/dl)	60.58±15.65	58.40±17.36	58.37±16.41	NS
TC (mg/dl)	207.78±32.47	207.40±34.81	205.44±36.39	NS

*<0.05 and †<0.01.
**P values are for comparisons with normal thyroid subjects after adjustment for appropriate confounding factors.*
Values are mean±standard deviation.
‡*Without treatment for hypertension;* §*without treatment for arrhythmia;* ¶*without treatment for diabetes mellitus;* ††*without treatment for hypertriglyceridemia;* ‖*without treatment for hyperlipidemia.*
ANOVA, analysis of variance. Other abbreviations see in Table 1.

thyroidism was higher than that in normal subjects (3.98% vs 1.41%) but the difference was not statistically significant. Subjects who had been diagnosed with AF were included with those with AF.

A total of 12% of subjects with SCH had high serum TSH levels (>10μU/ml). Subjects with SCH and TSH levels >10μU/ml were older than subjects with SCH and TSH levels =10μU/ml (67.28±9.62 years vs 71.07±9.70 years, $p<0.05$). Subjects with SCH and high TSH levels (>10μU/ml) did not have lower FBG, HbA1c or lipid levels compared with normal subjects (unpubl. data).

The correlation between laboratory data and fT3, fT4, and TSH levels in normal subjects is shown in Table3. Serum fT4 and fT3 levels in normal subjects were significantly associated with various laboratory data after adjusting for appropriate confounding factors.

We examined whether an association between IMT and thyroid dysfunction existed (Table4). There were weak associations between thyroid states and IMT-mean, and IMT-max, but these disappeared after adjusting for age and sex. Multiple logistic analysis did not detect a significant correlation between thyroid state (normal or subclinical

Table 3 Correlation Between Laboratory Data and Thyroid Hormone Level in Normal Subjects

Thyroid state	TG*†	FBG¶	HbA1c¶	TC‖	HDL‖	BMI	PR§	SBP‡	DBP‡
No. of subjects	3,084	2,976	2,976	2,726	2,726	3,130	3,057	2,355	2,355
TSH									
P	0.0015	NS	NS	0.015	0.0055	NS	NS	NS	NS
*	5.21	0.20	0.0090	1.83	-0.92	0.045	-0.10	-0.24	0.12
Normal thyroid									
fT3									
P	0.0043	NS	NS	NS	<0.0001	<0.0001	NS	NS	NS
*	12.16	0.15	-0.023	0.22	-4.01	1.06	0.12	1.75	0.64
fT4									
P	0.039	0.0006	0.030	0.0010	0.071	NS	<0.0001	0.002	0.023
*	18.00	6.03	0.14	13.09	3.16	0.43	4.83	7.06	2.95

*Correlation coefficient with each value.
P values are coefficient of correlation; p values are for comparisons with normal thyroid subjects after adjustment for appropriate confounding factors.
‡Without treatment for hypertension; §without treatment for arrhythmia; ¶without treatment for diabetes mellitus; ‖without treatment for hypertriglyceridemia; ‖without treatment for hyperlipidemia.
Abbreviations see in Table 1.

Table 4 Correlation Between Thyroid Status and IMT

	Normal thyroid	Hyperthyroidism	Hypothyroidism	Subclinical hyperthyroidism	Subclinical hypothyroidism	ANOVA p value
N	2,819	17	4	65	321	
IMT-max (mm)	1.30±0.55	1.21±0.63	1.73±0.97	1.25±0.53	1.40±0.58	0.048
Residual IMT-max*	0.002±0.491	-0.015±0.492	0.089±0.892	-0.052±0.467	-0.009±0.520	0.892
Residual IMT-max†	0.001±0.784	-0.046±0.506	0.094±0.855	-0.064±0.463	-0.001±0.510	0.786
N	2,818	17	4	65	320	
IMT-mean (mm)	0.821±0.13	0.79±0.13	0.93±0.09	0.83±0.13	0.83±0.13	0.042
Residual IMT-mean*	0.0004±0.105	-0.004±0.108	0.022±0.496	0.018±0.118	-0.008±0.112	0.581
Residual IMT-mean†	0.000±0.103	-0.301±0.103	0.014±0.035	0.014±0.111	-0.005±0.110	0.821

Values are mean ± standard deviation.
*Aadjusted for age and sex; †adjusted by age, BMI, SBP, FBS, number of cigarettes/day, TG, TC and HDL.
IMT, intima–media thickness. Other abbreviations see in Tables 1,2.

thyroid dysfunction) and the prevalence of atherosclerotic vascular diseases, such as cerebral infarction, transient cerebral ischemic attack, cerebral stroke, acute myocardial infarction and angina pectoris (p=0.090, unpubl. data).

Discussion

In this large cross-sectional study of subclinical thyroid dysfunction in Japanese subjects, although the prevalence of SCH increased with age overall, the prevalence of SCH in elderly patients was lower than that reported in other studies, particularly in women: 18.8% for men older than 75 years and 12.7% for women older than 75 years. This finding differs from that of previous studies in which the prevalence of SCH was higher in elderly women than in elderly men[1,3,6] In the Colorado study, the prevalence of SCH was 16% in men older than 75 years and 21% in women older than 75 years! The differences in these trends might be due to different genetic, ethnic or environmental backgrounds of the subjects. On the other hand, the prevalence of subclinical hyperthyroidism was 2.13% in our study, which is similar to that in the Colorado study (2.1%)!

We found that the presence of SCH was associated with lower FBG levels and that subclinical hyperthyroidism was associated with higher FBG and HbA1c levels. Moreover, our results also indicate that thyroid hormone levels in normal subjects are significantly associated with various laboratory data, including FBG and HbA1c levels. Compared with normal subjects, serum thyroid hormone levels in subjects with SCH were lower and levels in subjects with subclinical hyperthyroidism were higher. Therefore, lower or higher levels of thyroid hormone (within the normal range) in subclinical thyroid dysfunction might influence glucose metabolism.

We did not observe any significant association between subclinical thyroid dysfunction and lipid metabolism, which was consistent with a previous US study[5] However, other studies in Norway and Australia have reported dyslipidemia in subjects with SCH[6,7] We examined whether higher TSH levels (>10μU/ml) in subjects with SCH were associated with lipid metabolism, but did not find any significant association between lipid metabolism and TSH levels >10μU/ml in subjects with SCH compared with normal subjects. Moreover, higher TSH levels did not show any association with FBG levels. These results might be related to aging, because hypofunction of the endocrine glands occurs with age. Serum lipid, FBG, and HbA1c levels were sustained in subjects with high TSH levels and SCH.

We did not observe any significant association between subclinical thyroid dysfunction and IMT, which suggests that subclinical thyroid dysfunction might not be related to an increased risk of atherosclerosis. Moreover, we did not find any significant association between SCH and previous history of arteriosclerotic vascular diseases. However, this result was not consistent with previous studies[8,10] possibly because of the size of our study population and different