Original Article

Effects of the Y Chromosome on Cardiovascular Risk Factors in Japanese Men

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Excess cardiovascular risk in men compared with women has been suggested to be partly explained by effects of the Y chromosome. However, inconsistent results have been reported on the Y chromosome's genetic influence on blood pressure and lipid levels. The purpose of the present study was to settle the question whether genetic variants of the Y chromosome influence cardiovascular risk factors using a large epidemiological cohort, the Suita study. Possible influences of the Y chromosome polymorphisms (Y chromosome *A/u* insertion polymorphism [YAP], M175 and SRY+465) on cardiovascular risk factors were assessed in 974 Japanese men. The frequency of the YAP(+) allele in our study sample was 0.31. The prevalence of hypertension tended to be higher in YAP(+) than in YAP(−) men, and this tendency was found to be stronger among men aged 65 years or older. Men with the YAP(+) genotype had higher levels of high density lipoprotein (HDL) cholesterol compared with those with the YAP(−) genotype, even after adjustment for age, body mass index, and daily ethanol and cigarette consumption (57.0 ± 14.6 mg/dL vs. 54.2 ± 14.2 mg/dL, nominal $p=0.011$, adjusted $p=0.0062$). However, these observed nominal associations disappeared after adjusting for multiple testing (Bonferroni). No association was detected between the YAP genotype and myocardial infarction. Similarly, none of the associations with M175 and SRY+465 attained significance when multiple testing was taken into account. In conclusion, Y chromosome polymorphisms (YAP, M175 and SRY+465) do not appear to be associated with cardiovascular risk factors in Japanese men. Studies using much larger sample sizes and/or additional independent samples will be required for definitive conclusions. (*Hypertens Res* 2008; 31: 1687–1694)

Key Words: Y chromosome, polymorphism, risk factors

Introduction

Higher mortality rates from cardiovascular disease in men than in women are consistent findings among studies of different populations including the Japanese (1–3), and male gender has been recognized as an important risk factor. The greater risk associated with male gender may be partly due to sex-related differences in the prevalence of hypertension (4,

5) and/or dyslipidemia (6). Although the level of significance was not given, in a study of 8,168 Japanese subjects undergoing general health screening, the mean levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), low density lipoprotein (LDL), cholesterol, and triglyceride (TG) were all higher in men ($n=5,244$) than in women ($n=2,924$) (7). In that study, mean high density lipoprotein (HDL) cholesterol levels were lower in men than in women. Sex-related differences in HDL cholesterol levels were also evident among eld-

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erly Japanese patients with coronary artery disease (CAD), with men having significantly lower levels of HDL cholesterol compared with women (6).

It has been hypothesized that sex-related differences in cardiovascular risk can be accounted for by the effect of Y chromosome. Analyses of the Y chromosome consomic strains derived from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) revealed the possible effect of the Y chromosome on determination of blood pressure (BP), with an SHR Y chromosome in a WKY genetic background exerting the BP-elevating effect (8–10). Similarly, replacement of the SHR Y chromosome with that of Brown Norway rats has been associated with significant reduction in both SBP and DBP (11).

In line with this view, studies on genetic variations in the non-recombining region of the Y chromosome have been undertaken in relation to BP. *HindIII* restriction fragment length polymorphism (12) is one of the most widely studied variants of the Y chromosome. Australian men negative for the *HindIII* restriction site have been shown to have significantly higher DBP than *HindIII* positive men (13), whereas a study based on Polish and Scottish men reported an association between the presence of the *HindIII* restriction site and elevated levels of SBP and DBP (14). The presence of the *HindIII* restriction site was more prevalent among hypertensive than normotensive subjects (15). Men with *HindIII*(–) genotype had significantly higher levels of LDL and total cholesterol (TC) than those with *HindIII*(+) genotype (16). In contrast, no association was found between *HindIII*(+/-) polymorphism and BP, presence of hypertension, or lipid levels in three populations of white European origins (17). In a more recent study of young men, absence of *HindIII* restriction site was associated with higher SBP and DBP (18).

While previous association studies conducted predominantly in Caucasians have produced conflicting results regarding the effect of *HindIII* genotype on BP and lipid levels, data on Y chromosome polymorphisms in a Japanese population are scarce. One such study carried out in the Aomori population demonstrated the association of Y chromosome *Alu* insertion polymorphism (YAP) at DYS287 with higher HDL cholesterol levels, but not with hypertension (19). In the Japanese, an *Alu* insertion polymorphism on the Y chromosome has been reported to be a paternal marker to distinguish two major lineages, the Jomon (YAP-positive) and Yayoi (YAP-negative) (20). It remains to be determined whether polymorphisms within the male-specific region of the Y chromosome confer excess cardiovascular risk in men. Therefore, the aim of the present study was to examine whether Y chromosome polymorphic markers (YAP, M175 and SRY+465) in Japanese men were associated with myocardial infarction (MI), atherosclerosis, and cardiovascular risk factors including body mass index (BMI), BP, lipids, and glucose.

Methods

Study Population

The selection criteria and design of the Suita study have been described previously (21–27). Male subjects under 85 years of age who were recruited consecutively into the Suita study ($n=974$) were included in the present analysis. The MI case group consisted of 322 randomly selected inpatients and outpatients with documented MI who were enrolled in the Division of Cardiology at the National Cardiovascular Center. Both controls and MI cases were of the same ethnicity (Japanese) and recruited from the National Cardiovascular Center. Only those who gave written informed consent were included in the study. The study protocol was approved by the Ethics Committee of the National Cardiovascular Center and the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center.

Subjects with SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, and/or those currently using antihypertensive medication were classified as having hypertension (HTN). Subjects who smoked at the time of study entry were categorized as smokers. The definition of type 2 diabetes were based on fasting blood glucose levels ≥ 126 mg/dL, HbA1c $\geq 6.5\%$, and/or current treatment for diabetes. 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 (21). BMI was calculated as body weight (kg) divided by height squared (m^2). Waist-to-hip (*W/H*) ratio was calculated as the waist (cm) divided by the hip (cm).

Genotyping

The YAP genotype was determined by electrophoretic patterns of the amplified products using a previously reported primer pair (20): a 455-bp (YAP-positive) or a 150-bp fragment (YAP-negative). Initially, the YAP polymorphism was genotyped both in the Suita study ($n=974$) and MI case group ($n=322$). An additional 200 subjects were then genotyped for confirmation of the significance of the YAP polymorphism. Genotyping for SRY+465 (28) and M175 (29) was performed on 7900HT (Applied Biosystems, Foster City, USA) by the TaqMan method according to the manufacturer's instructions. Genotyping for SRY+465 and M175 was carried out only in the Suita study ($n=974$).

Statistical Analysis

Values are expressed as mean $\pm$ SD. Simple correlation analyses were performed to determine the association between Y chromosome polymorphisms (YAP, SRY+465 and M175) and risk factors. Residuals of height, SBP, DBP, IMT, HDL cholesterol, fasting blood glucose, and glycated HbA1c levels were calculated with adjustment for covariates. One way

Table 1. Characteristics of the Study Population

	Suita study	MI subjects	<i>p</i> value
Number	974	322	—
YAP(+) (<i>n</i> (%))	291 (31.0) ^a	97 (30.1)	n.s.
YAP(+) (<i>n</i> (%))	278 (30.5) ^b	97 (30.1)	n.s.
SRY+465(T) (<i>n</i> (%))	277 (29.7) ^c	—	—
M175(+) (<i>n</i> (%))	437 (46.8) ^d	—	—
Age (year)	65.95±10.47	57.40±9.69	<0.0001
BMI (kg/m ²)	23.39±2.93	23.82±2.85	0.019
HTN (<i>n</i> (%))	400 (41.1)	158 (49.1)	0.041
TG (mg/dL)	120.6±79.6	—	—
TC (mg/dL)	199.2±31.9	—	—
Current smokers (<i>n</i> (%))	270 (27.7)	213 (66.1)	<0.0001
MI (<i>n</i> (%))	26 (2.7)	322 (100)	—

Data are presented as mean±SD. ^a*n*=938, ^b*n*=912 without coronary artery disease, ^c*n*=932, ^d*n*=934. n.s., not significant; YAP, Y chromosome *Alu* insertion polymorphism; BMI, body mass index; HTN, prevalence of hypertension as defined by SBP≥140 mmHg, DBP≥90 mmHg and/or current use of antihypertensive medication; TG, triglyceride; TC, total cholesterol; MI, myocardial infarction.

Table 2. Sequences of Primers and Probes Used for Genotyping of Y Chromosome Polymorphisms

Polymorphism	Probe		Primer	
	VIC	FAM	Forward	Reverse
YAP			cagggaagataaagaata	aagccactattagacaacct
SRY+465	tgcacttcgctgcaga	tgcacttcactgcagag	agatgctgccgaagaattgc	tagctggtgctccattcttgagt
M175	acttctcttcaagaat	ctacttcaagaatgaa	ctcaactccagtcatttaactctctg	catgtactttgtccaatgctgaaa

analysis of variance (ANOVA) was used to test whether there was a difference in continuous variables between the groups. Group differences in categorical variables were assessed by the χ^2 test. Multiple logistic regression analyses were performed to obtain predictors for MI. The level of significance was adjusted for multiple testing by a Bonferroni correction: *p* values were multiplied by 57 (19 phenotypes and 3 genotypes). Statistical analyses were performed with the JMP statistical package (SAS Institute Inc., Cary, USA) and Sample Power 2 (SAS Institute Inc.).

Results

Characteristics of the Study Populations

Clinical characteristics of male subjects in the Suita study (*n*=974) and MI case group (*n*=322) are presented in Table 1. Subjects in the MI group were significantly younger and had higher BMI than those in the Suita study. MI cases were also characterized as having a higher prevalence of smokers and hypertension compared with those in the Suita study. We analyzed three Y chromosome polymorphisms (YAP, SRY+465, and M175) in our Japanese sample. Sequences of primers and probes used for genotyping are shown in Table 2. The frequency of the YAP(+) in MI subjects (30.1%) was not different from that in the Suita study (31.0%) (Table 1). The

difference remained non-significant when comparisons of the YAP(+) frequency were made between the Suita study without MI subjects (*n*=912) and the MI group (*n*=322). The prevalence of SRY+465(T) and M175(+) in the Suita study was 29.7% and 46.8%, respectively.

Y Chromosome Polymorphism and Cardiovascular Risk Factors

Table 3 shows the association analysis between YAP polymorphism and cardiovascular risk factors. Levels of HDL cholesterol were significantly higher in YAP(+) than YAP(−) men (57.0±14.6 mg/dL vs. 54.2±14.2 mg/dL, nominal *p*=0.011). The association of the YAP(+) genotype with higher HDL cholesterol remained significant even after controlling for age, BMI, and daily ethanol and cigarette consumption. Men in the YAP(−) group were taller than those in the YAP(+) group (165.6±6.0 cm vs. 164.7±5.9 cm, nominal *p*=0.026, age-adjusted *p*=0.032). Hypertension tended to be more prevalent in the YAP(+) group than in the YAP(−) group (47.2% vs. 40.7%, nominal *p*=0.066). When the analysis was restricted to those aged 65 years or older, the prevalence of hypertension was significantly higher in the YAP(+) group than in the YAP(−) group (59.7% vs. 48.6%, nominal *p*=0.014). However, we did not find any significant association between the YAP genotypes and BP levels among the

Table 3. Association between YAP Polymorphism and Cardiovascular Risk Factors

	YAP(−) (<i>n</i> =647)	YAP(+) (<i>n</i> =291)	Nominal <i>p</i>	Corrected <i>p</i>
Age (year)	65.8±10.4	66.3±10.6	n.s.	n.s.
BMI (kg/m ²)	23.4±2.9	23.4±3.1	n.s.	n.s.
<i>W/H</i> ratio	0.93±0.05	0.93±0.05	n.s.	n.s.
HTN (%)	40.7	47.2	0.066	n.s.
Antihypertensive drugs (%)	28.9	31.4	n.s.	n.s.
Height (cm)	165.6±6.0	164.7±5.9	0.026	n.s.
Res, Height ^a	0.28±5.26	−0.53±5.50	0.032	n.s.
TC (mg/dL)*	198.3±32.3	201.8±31.1	n.s.	n.s.
TG (mg/dL)*	123.0±87.2	111.7±63.9	0.067	n.s.
HDL cholesterol (mg/dL)*	54.2±14.2	57.0±14.6	0.011	n.s.
Res, HDL cholesterol ^{a,b}	−0.83±13.1	1.89±12.9	0.0062	n.s.
SBP (mmHg)**	122.3±16.5	123.9±18.2	n.s.	n.s.
Res, SBP ^{a,c}	−0.5±15.9	1.1±17.3	n.s.	n.s.
DBP (mmHg)**	77.1±10.0	77.0±10.3	n.s.	n.s.
Res, DBP ^{a,d}	0.0±9.5	−0.1±10.1	n.s.	n.s.
Glucose (mg/dL)***	100.4±21.7	101.9±21.3	n.s.	n.s.
HbA1c (%)***	5.51±0.75	5.53±0.77	n.s.	n.s.
HTN (%) [†]	28.9	25.9	n.s.	n.s.
HTN (%) [‡]	48.6	59.7	0.014	n.s.

Data are presented as mean±SD. *Subjects without medication for dyslipidemia were included: 580 men for YAP(−) and 248 men for YAP(+). **Subjects without the use of antihypertensive drugs were included: 460 YAP(−) and 200 YAP(+) men. ***Subjects who were not receiving treatment for diabetes were included: 601 YAP(−) and 270 YAP(+) men. [†]Subjects younger than 65 years of age were included: 260 YAP(−) and 108 YAP(+) men. [‡]Subjects older than 65 years of age were included: 387 YAP(−) and 183 YAP(+) men. Covariates used to calculate residual values were ^aage, ^bage, BMI, ethanol consumption (g/d) and number of cigarettes per day, ^cage and BMI, ^dage-squared and BMI. n.s., not significant; Res, residual; BMI, body mass index; *W/H* ratio, waist-to-hip ratio; HTN, prevalence of hypertension as defined by SBP≥140 mmHg, DBP≥90 mmHg and/or the current use of antihypertensive medication; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure.

older subjects who were not on treatment for hypertension: 128.7±18.6 mmHg and 124.5±16.8 mmHg for YAP(+) and YAP(−) men, respectively (nominal *p*=0.12). In order to evaluate nominal associations observed in Table 3, an additional 200 male subjects were included in the study population. However, the increase in sample size rather weakened the above mentioned associations: nominal *p*=0.045 for height, *p*=0.038 for age-adjusted height, nominal *p*=0.02 for HDL cholesterol, and *p*=0.02 for HDL cholesterol adjusted for BMI and ethanol and cigarette consumption. In the analysis of a larger sample of men (*n*=1,138), the prevalence of hypertension between the YAP(−) and YAP(+) groups was no longer statistically different (40.6% vs. 45.5%, nominal *p*=0.12).

As summarized in Table 4, male subjects with SRY+465(T) tended to have higher levels of glucose (102.9±28.1 mg/dL vs. 100.1±18.3 mg/dL, nominal *p*=0.078, age and BMI adjusted *p*=0.050) and HbA1c (5.59±0.91% vs. 5.49±0.68%, nominal *p*=0.063, age and BMI adjusted *p*=0.052) compared with subjects with SRY+465(C). TG levels tended to be lower in M175(+) positive men than in M175(−) men; 113.1±69.0 mg/dL vs. 125.2±89.7 mg/dL, nominal *p*=0.032 (Table 5). However, it

should be noted that none of the significant or marginal associations observed between Y chromosome polymorphisms (YAP, SRY+465 and M175) and cardiovascular risk factors remained significant after correction for multiple testing (Bonferroni).

Haplotype analyses were then performed to examine a possible influence of different patterns of Y chromosomal polymorphisms (YAP, SRY+465 and M175) on hypertension and HDL cholesterol levels. Four common haplotypes were identified in our Japanese sample: YAP(+)M175(+)SRY465(C), YAP(−)M175(−)SRY465(T), YAP(−)M175(+)SRY465(C), and YAP(−)M175(−)SRY465(C). As shown in Table 6, no significant difference was observed in the prevalence of hypertension and HDL cholesterol levels between the haplotype groups. We did not find any significant association between haplotypes and the prevalence of hypertension even when separate analysis was conducted for older or younger men using an age cut off of 65 years (data not shown).

YAP Polymorphism and Atherosclerosis

We examined the effect of YAP polymorphisms on the marker of atherosclerosis as measured by mean carotid IMT.

Table 4. Association between SRY+465 Polymorphism and Cardiovascular Risk Factors

	SRY+465(C) (n=655)	SRY+465(T) (n=277)	Nominal <i>p</i>	Corrected <i>p</i>
Age (year)	65.9±10.5	66.2±10.4	n.s.	n.s.
BMI (kg/m ²)	23.4±3.0	23.3±2.7	n.s.	n.s.
<i>W/H</i> ratio	0.93±0.05	0.93±0.05	n.s.	n.s.
HTN (%)	43.5	40.6	n.s.	n.s.
Antihypertensive drugs (%)	29.8	29.8	n.s.	n.s.
Height (cm)	165.1±5.9	165.7±6.0	n.s.	n.s.
Res, Height ^a	-0.15±5.39	0.43±5.3	n.s.	n.s.
TC (mg/dL)*	199.6±32.5	199.2±30.6	n.s.	n.s.
TG (mg/dL)*	120.5±80.7	117.4±81.6	n.s.	n.s.
HDL cholesterol (mg/dL)*	55.3±14.3	54.7±14.5	n.s.	n.s.
Res, HDL cholesterol ^{a,b}	0.11±13.0	-0.24±13.3	n.s.	n.s.
SBP (mmHg)**	123.2±16.7	122.1±17.6	n.s.	n.s.
Res, SBP ^{a,c}	0.3±16.1	-0.6±16.6	n.s.	n.s.
DBP (mmHg)**	77.4±10.0	76.5±10.1	n.s.	n.s.
Res, DBP ^{a,d}	0.3±9.6	-0.5±9.6	n.s.	n.s.
Glucose (mg/dL)***	100.1±18.3	102.9±28.1	0.078	n.s.
Res, Glucose ^{a,c}	17.7±0.7	27.9±1.8	0.050	n.s.
HbA1c (%)***	5.49±0.68	5.59±0.91	0.063	n.s.
Res, HbA1c ^{a,c}	0.68±0.03	0.90±0.06	0.052	n.s.

Data are presented as mean±SD. *Subjects without medication for dyslipidemia were included: 577 men for SRY+465(C) and 245 men for SRY+465(T). **Subjects without the use of antihypertensive drugs were included: 457 and 199 men for SRY+465(C) and SRY+465(T), respectively. ***Subjects who were not receiving treatment for diabetes were included: 610 men with SRY+465(C) and 255 men with SRY+465(T). Covariates used to calculate residual values were ^aage, ^bethanol consumption (g/d) and number of cigarettes per day, ^cage and BMI, ^dage-squared and BMI. See Table 3 for abbreviations.

Table 5. Association between M175 Polymorphism and Cardiovascular Risk Factors

	M175(-) (n=498)	M175(+) (n=436)	Nominal <i>p</i>	Corrected <i>p</i>
Age (year)	65.9±10.5	66.1±10.5	n.s.	n.s.
BMI (kg/m ²)	23.4±2.8	23.4±3.0	n.s.	n.s.
<i>W/H</i> ratio	0.93±0.05	0.93±0.05	n.s.	n.s.
HTN (%)	40.4	45.4	n.s.	n.s.
Antihypertensive drugs (%)	30.2	28.3	n.s.	n.s.
Height (cm)	165.5±5.9	165.1±6.0	n.s.	n.s.
Res, Height ^a	0.20±5.16	-0.20±5.56	n.s.	n.s.
TC (mg/dL)*	199.0±31.1	199.8±33.0	n.s.	n.s.
TG (mg/dL)*	125.2±89.7	113.1±69.0	0.032	n.s.
HDL cholesterol (mg/dL)*	54.5±14.1	55.8±14.6	n.s.	n.s.
Res, HDL cholesterol ^{a,b}	-0.57±13.1	0.65±13.1	n.s.	n.s.
SBP (mmHg)**	121.7±16.4	124.0±17.6	0.09	n.s.
Res, SBP ^{a,c}	-1.0±15.7	1.1±17.0	0.094	n.s.
DBP (mmHg)**	76.8±9.9	77.4±10.3	n.s.	n.s.
Res, DBP ^{a,d}	-0.2±9.3	0.2±10.0	n.s.	n.s.
Glucose (mg/dL)***	101.1±23.8	100.6±18.9	n.s.	n.s.
HbA1c (%)***	5.53±0.81	5.49±0.68	n.s.	n.s.

Data are presented as mean±SD. *Subjects without medication for dyslipidemia were included: 441 men for M175(-) and 382 men for M175(+). **Subjects without the use of antihypertensive drugs were included: 350 M175(-) and 306 M175(+) men. ***Subjects who were not receiving treatment for diabetes were included: 468 M175(-) and 399 M175(+) men. Covariates used to calculate residual values were ^aage, ^bethanol consumption (g/d) and number of cigarettes per day, ^cage and BMI, ^dage-squared and BMI. See Table 3 for abbreviations.

Table 6. Association of Haplotypes with Prevalence of Hypertension and HDL Cholesterol in Japanese Men

	<i>n</i> (%)	HTN (%)	χ^2		HDL cholesterol (mg/dL)	ANOVA	
			Nominal <i>p</i>	Corrected <i>p</i>		Nominal <i>p</i> *	Corrected <i>p</i> *
YAP(+)M175(+)/SRY+465(C)	288 (31.0)	47.2	0.30	n.s.	55.5±14.5	0.049	n.s.
YAP(-)M175(-)/SRY+465(T)	275 (29.6)	40.7			54.3±14.2		
YAP(-)M175(+)/SRY+465(C)	148 (15.9)	41.9			53.5±14.6		
YAP(-)M175(-)/SRY+465(C)	219 (23.5)	39.7			51.2±13.3		

Data are presented as mean±SD. *Adjustment was made for age, BMI, ethanol consumption (g/d) and number of cigarettes per day. n.s., not significant; HDL, high-density lipoprotein; HTN, hypertension; BMI, body mass index.

Irrespective of whether crude values or residual values adjusted for age, BMI, SBP, glucose, number of cigarettes per day, TG, TC, and HDL cholesterol were used for analysis, no difference in mean IMT was observed between YAP(+) men (*n*=274) and YAP(-) men (*n*=606): mean IMT (mm) was 0.810±0.125 mm for the YAP(-) group and 0.809±0.137 mm for the YAP(+) group.

YAP Polymorphism and MI

The association between YAP polymorphism and MI was investigated in MI cases (*n*=322) and subjects in the Suita study who were free from CAD (*n*=912). Multiple logistic regression analysis did not find any correlation between YAP(+/-) polymorphism and MI. The predictors for MI included age (*p*<0.0001), smoking habit (*p*<0.0001), and the presence of diabetes or hyperglycemia as defined by glucose levels of ≥126 mg/dL (*p*<0.0001).

Discussion

The sex-related difference in mortality rate from cardiovascular disease has been consistently demonstrated, with male gender being associated with increased risk (1–3). Results based on the chromosome Y consomic strains derived from SHR and WKY rats have suggested the possible effect of Y chromosome on determination of BP (8–11). It can be speculated that sexual dimorphism in cardiovascular risk can be partly explained by the effect of the Y chromosome, and polymorphisms within the male specific non-recombining region of the Y chromosome are linked to increased cardiovascular risk in men.

We examined the effect of Y chromosome polymorphisms (YAP, SRY+465, and M175) on cardiovascular risk factors in our Japanese sample. The frequency of the YAP(+) allele was 31.0% in the present study, which is comparable to that observed in Shizuoka (33%) (20) and Aomori (34%) (19) but different from that of Okinawa (56%) (20). The previously reported prevalence of M175(+) and SRY+465(T) in Japanese subjects was 45.4% and 25.9%, respectively (30). Similarly, the prevalence of M175(+) was 46.7%, and that of SRY+465 (T) was 29.7%, in our study sample.

In the present analysis, the prevalence of hypertension tended to be higher in YAP(+) than in YAP(-) men, and this tendency was found to be stronger among men aged 65 years or older. However, the association was no longer significant after controlling for multiple testing. In agreement with a previous investigation reporting the association of YAP polymorphism with HDL cholesterol in Japanese (19), we observed a similar tendency of YAP(+) subjects to have higher levels of HDL cholesterol compared with YAP(-) subjects even after adjustment for age, BMI, and daily ethanol and cigarette consumption. However, the observed association disappeared when the level of significance was adjusted for multiple testing.

We examined whether YAP(+/-) polymorphism was associated with mean carotid IMT, known as an excellent noninvasive marker of atherosclerosis. No association was detected between YAP and IMT. These results suggest that the influence of YAP polymorphism on the development of atherosclerosis, possibly through its effect on BP levels, might be lowered due to the tendency of YAP(+) men to have higher HDL cholesterol levels.

The YAP(+) allele was not associated with MI, and the prevalence of YAP(+) subjects in the MI group was not different from that in the Suita study. We estimated that our sample had 80% statistical power to detect an odds ratio of 1.5 for MI. The sample size of our study might be sufficient to detect a significant association between YAP and MI. However, it is possible that an association between YAP and MI can be lost at an odds ratio of 1.3 due to the limited sample power (0.45).

Previous studies on the effect of the *Hind*III polymorphism in the non-recombining region of the Y chromosome on BP and lipid levels have produced discrepant results. Since genotyping of the *Hind*III polymorphism was not performed in our study sample, a direct comparison is not possible, which is one of the limitations of the present study.

The possible association between Y chromosome polymorphism and height is noteworthy. We observed a nominal association of YAP(+/-) polymorphism with height and prevalence of hypertension in our study sample. In a study of 409 Australian Caucasian men, *Hind*III negative men were taller than *Hind*III positive men: 174.7±6.6 cm vs. 172.8±7.7 cm, *p*=0.009 (31). Absence of the *Hind*III restriction site was

found to be associated not only with younger age at peak height velocity but also with higher SBP and DBP levels before and after pubertal growth, suggesting the possible role of earlier exposure to androgen among the *HindIII* negative males in the development of hypertension (18). Previous studies in animal models may support this notion. In male SHR, the increase in blood pressure has been shown to be suppressed by treatment with androgen receptor antagonist or castration (32, 33). Further research is required to clarify the role of the Y chromosome in androgen-mediated hypertension.

In conclusion, the Y chromosome polymorphisms genotyped in 974 Japanese men (YAP, SRY+465, and M175) showed nominal associations with height, lipid levels, and the prevalence of hypertension, none of which remained significant after adjustment for multiple testing. The contribution of the YAP polymorphism to height, HDL cholesterol, and the prevalence of hypertension appears to be small, if even present, and it could not be reliably detected in our current sample size. Much larger sample sizes and/or additional independent samples will be required to make definitive conclusions.

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