Table 5. Comparison of Hypertension Prevalence by Genotypes of Three Polymorphisms of *XDH* in a Japanese General Population by Sex

SNP	Genotype group	Women		Men	
		Odds ratio (95% CI)	p*	Odds ratio (95% CI)	p*
47686C>T	CC	1		1	
[CC/CT/TT=815/819/244]	CT+TT	0.90 (0.68–1.20)	0.469	1.10 (0.83–1.46)	0.521
	CC+CT	1		1	
	TT	1.04 (0.67–1.62)	0.861	1.52 (1.01–2.29)	0.047
67873A>C	AA	1		1	
[AA/AC/CC=1,720/154/5]	AC+CC	0.97 (0.58–1.61)	0.906	1.84 (1.11–3.06)	0.018
	AA+AC	1		1	
	CC	0.62 (0.05–7.33)	0.704	3.98 (0.20–80.72)	0.368
69901A>C	AA	1		1	
[AA/AC/CC=1,372/463/42]	AC+CC	1.30 (0.95–1.78)	0.099	1.11 (0.80–1.53)	0.530
	AA+AC	1		1	
	CC	0.96 (0.40–2.35)	0.936	3.14 (1.06–9.27)	0.039

*Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) for hypertension. *XDH*, xanthine dehydrogenase gene; SNP, single nucleotide polymorphism; CI, confidence interval; [ ], sample numbers of three kinds of genotypes.

on ba-PWV were available for patient 2.

Associations of 11 Variations with Hypertension in the General Population

Three missense mutations (G172R, A932T, and N1109T) and eight common SNPs (11488C>G, 37387A>G, 44408A>G, 46774G>A, 47686C>T, 49245A>T, 66292C>G, and 69901A>C) were used for the association studies in the case-control setting for men and woman separately. Adjusted for age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking), a logistic regression analysis of the case-control study showed that three of the eight SNPs were significantly associated with hypertension in men: TT *vs.* CC+CT for 47686C>T (exon 22, OR: 1.52, p=0.045) and CC *vs.* AC+AA for 69901A>C (intron 31, OR: 3.14, p=0.039) in the recessive model, and AC+CC *vs.* AA for 67873A>C (N1109T) (exon 31, OR: 1.84, p=0.018) in the dominant model (Table 5).

SBP was 2.44 mmHg higher in women with the AC+CC genotype of the positively associated SNP 69901A>C in *XDH* than in women with the AA genotype (p=0.037). Although there was no significant difference in SBP or DBP between the AC+CC and AA genotypes of 69901A>C in men, DBP was 4.18 mmHg higher in men with the CC genotype of 69901A>C than in men with the AA+AC genotype (p=0.088). DBP was 2.75 mmHg higher in men with the AC+CC genotype of the positively associated SNP 67873A>C than in men with the AA genotype (p=0.021) (Table 6).

Regarding the three missense mutations, there were 6 subjects with a homozygote allele in *XDH* G172R and 5 subjects with one in N1109T, but no subjects with one in A932T. The subjects with a homozygote allele in G172 and N1109T did not have any specific clinical characteristics (data not shown).

Association of 11 Variations with Carotid Atherosclerosis in Hypertensive Subjects

Three missense mutations (G172R, A932T, and N1109T) and eight common SNPs (11488C>G, 37387A>G, 44408A>G, 46774G>A, 47686C>T, 49245A>T, 66292C>G, and 69901A>C) were tested for associations with carotid atherosclerosis in patients with essential hypertension. After the full adjustment for all confounding factors (age, BMI, SBP, DBP, current smoking status, alcohol consumption, and presence of diabetes mellitus and dyslipidemia), only one polymorphism (69901A>C) was found to be independently associated with carotid atherosclerosis in the dominant model (χ^2=4.82, p=0.028). Other factors—age (χ^2=67.70, p<0.001), SBP (χ^2=15.11, p<0.001), and DBP (χ^2=4.28, p=0.039)—were related to carotid atherosclerosis. We compared IMT and ba-PWV values among the alleles in *XDH* 69901A>C. There were no significant differences between alleles in either IMT or ba-PWV. However, ba-PWV values tended to differ significantly (AA: 1,794, AC: 1,825, CC: 2,024 cm/s, p=0.075) in *XDH* 69901A>C. These findings may indicate that hypertensive patients with the CC of *XDH* 69901A>C are more susceptible to atherosclerosis than those with the A allele.

Associations of 11 Variations with Chronic Kidney Disease in Hypertensive Subjects

We divided the essential hypertensive patients into two groups using a cutoff estimate of Ccr 60 mL/min. The CKD group (Ccr <60 mL/min) showed significantly higher age

Table 6. Multivariate-Adjusted Blood Pressure Levels on Genotypes of Three SNPs of *XDH* by Sex

SNP	Genotype group	Women				Men			
		SBP, mmHg	p*	DBP, mmHg	p*	SBP, mmHg	p*	DBP, mmHg	p*
47686C>T	CC	127.60±0.79	0.752	79.69±0.45	0.630	131.73±0.89	0.976	79.58±0.51	0.707
	CT+TT	127.93±0.69		76.40±0.39		131.76±0.78		79.83±0.45	
	CC+CT	127.84±0.56	0.782	76.69±0.31	0.138	131.68±0.63	0.779	79.60±0.36	0.393
	TT	127.40±1.50		75.34±0.85		132.16±1.57		80.44±0.90	
67873A>C	AA	127.87±0.54	0.538	76.48±0.31	0.546	131.50±0.61	0.178	79.48±0.35	0.021
	AC+CC	126.70±1.83		77.13±1.04		134.30±1.99		82.23±1.14	
	AA+AC	127.82±0.52	0.108	76.55±0.30	0.375	131.77±0.59	0.441	79.73±0.34	0.425
	CC	112.54±9.48		71.76±5.38		122.35±12.19		74.14±7.00	
69901A>C	AA	127.10±0.61	0.037	76.33±0.35	0.290	131.23±0.68	0.136	79.37±0.39	0.079
	AC+CC	129.54±0.99		77.03±0.56		133.23±1.15		80.72±0.66	
	AA+AC	127.87±0.53	0.253	76.58±0.30	0.289	131.72±0.59	0.689	79.64±0.34	0.088
	CC	124.04±3.30		74.57±1.87		133.43±4.23		83.82±2.42	

Data are mean±SD. *Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) for hypertension. SNP, single nucleotide polymorphism; *XDH*, xanthine dehydrogenase gene; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 7. Comparison of Chronic Kidney Disease Prevalence by Genotypes of 66292 C>G in *XDH* in Hypertensives by Sex

Genotype group	Men [CC/CG/GG=11/123/363]		Women [CC/CG/GG=11/83/315]	
	Odds ratio (95% CI)	p*	Odds ratio (95% CI)	p*
CG+GG	1	0.5545	1	0.1093
CC	1.51 (0.369–5.924)		3.48 (0.725–16.412)	
GG	1	0.0006	1	0.5617
CC+CG	2.36 (1.348–3.850)		1.18 (0.663–2.084)	

*Multiple logistic regression analysis, adjusted for age, body mass index, diabetes mellitus, systolic blood pressure, and diastolic blood pressure. *XDH*, xanthine dehydrogenase gene; [ ], sample numbers of three kinds of genotypes; CI, confidence interval.

($p<0.001$), lower BMI ($p<0.001$), and lower DBP ($p<0.001$) than the non-CKD group.

As shown in Table 7, after adjustment for age, BMI, SBP, DBP, and the number of patients that suffer from diabetes mellitus, logistic regression analysis showed that one SNP (66292C>G) of the 11 variations was strongly associated with chronic kidney disease in the recessive model in men (OR=2.36, $p=0.0006$). This significant association was still positive after a Bonferroni correction ($p=0.0006<0.05/11$). However, there was no significant difference in Ccr value between GG and CC+CG in *XDH* 66292C>G in male hypertensive patients (GG: 84.73±39.14 *vs.* CC+CG: 80.32±73.26 mL/min, $p=0.384$).

Discussion

The present study is the first to examine the relationships between genetic variations in *XDH* and hypertension or its complications in human. After the screening for possible genetic variations in the promoter and all exon regions of *XDH* in 48 patients with hypertension, 11 variations, including 3 missense mutations and 8 common SNPs, were genotyped and used to assess the roles of these genetic changes in hypertension in a large population of hypertensive subjects and in a general population. The 4 hypertensive patients with a rare missense mutation (G172R or N1109T) in homozygous form had hypertension. More importantly, 67873A>C (N1109T) also showed a positive association with hypertension in men in a multivariable logistic analysis. In addition, DBP was 2.75 mmHg higher in men with the AC+CC genotype of 67873A>C than in men with the AA genotype ($p=0.021$). This indicates that 67873A>C may be a functional risk factor for hypertension in males. Another two SNPs, 47686C>T in the exon region and 69901A>C in the intron region, were also found to be significantly related to hypertension in men. Furthermore, SBP was 2.44 mmHg higher in women with the AC+CC genotype of 69901A>C than those with the AA genotype ($p=0.037$). Since a significant association was obtained in the multivariable analysis with adjustment for confounding risk factors, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (current smoking and drinking) by sex, these

three SNPs appear to be independent risk factors for hypertension. The C allele of 69901A>C was associated with greater susceptibility in male subjects. In females, there was a significant association between 69901A>C and blood pressure. Although there was no significant difference in SBP or DBP between the AC+CC and AA genotypes of 69901A>C in men, DBP was 4.18 mmHg higher in men with the CC genotype of 69901A>C than in men with the AA+AC genotype ($p=0.088$). Taking these findings together, we speculate that, among males, those with 67873A>C (N1109T) were most susceptible to hypertension.

This is also the first report to show a positive relationship between SNPs of *XDH* and CKD in hypertensive patients. It is well reported that age, sex, blood pressure, BMI, and diabetes mellitus are all factors in renal dysfunction (*38–41*). Our results also showed that age, DBP, and BMI differed significantly between hypertensive patients with Ccr <60 mL/min and those with Ccr ≥60 mL/min. But no significant difference in SBP or the number of diabetes mellitus patients was found with or without CKD in these hypertensive subjects. After adjustment for age, sex, BMI, SBP, DBP, and the number of patients having diabetes mellitus, the logistic regression analysis showed that only one SNP (66292C>G) was strongly associated with CKD in hypertensive patients. This indicates that 66292C>G may be an independent risk factor for CKD in hypertensive patients.

SNP 69901A>C was found to be significantly associated with carotid atherosclerosis in hypertensive patients in our study. Although we did not find a significant difference between genotypes in any of the various atherosclerotic variables, hypertensive patients with the A allele of 69901A>C tend to be more susceptible to atherosclerosis than those with the C allele.

How the SNPs of *XDH* influence the pathogenesis of hypertension and its complications, including atherosclerosis and CKD, remains unclear. Among the four SNPs that showed a positive association with hypertension or with atherosclerosis and CKD in hypertensive patients, 67873A>C and 47686C>T are in exon regions, and 69901A>C and 66292C>G are in intron regions. 67873A>C causes a missense mutation in exon 31, leading to an amino acid substitution from Asn to Thr at position 1109. But 47686C>T does not result in a change in amino acids. In addition, the three missense mutations, 26390G>A (G172R), 64606G>A (A932T), and 67873A>C (N1109T), occurred in highly conserved residues among different species, all resulting in a hydrophilic amino acid substitution, which may influence reactive centers of enzymes. The XDH protein consists of three functional subunit domains, each of which binds a different cofactor, from amino acids 1 to 165 for binding $2Fe_2S_2$, from 226 to 531 for binding flavin adenine dinucleotide, and from 590 to 1332 for binding molybdoptern (Mo-Co) (*5*). The missense mutation G172R is not in the predicted functional domain, but A932T and N1109T are in the domain for binding molybdoptern. A932T and N1109T are not in the domain for binding flavin adenine dinucleotide, which is thought to play a major role in the conversion of XDH to XO and which increases ROS production in some pathological conditions, including hypertension and atherosclerosis (*5*). However, it is important to note a recent report that XOR has both inorganic nitrate reductase and nitrite reductase activity at its Mo-Co site (*42, 43*). This implies that an amino acid mutation at the Mo-Co site may influence nitric oxide production and modulate ROS production. Those four hypertensive patients with A932T and N1109T in the homozygous form all had high blood pressure, N1109T showed significant associations with hypertension and blood pressure, and the Mo-Co–binding site is the most conserved region of *XDH* among human, rat, and mouse (*44*). This strongly indicates that the mutations A932T and N1109T may be functional risk factors for hypertension. Further *in vivo* and *in vitro* studies are needed to clarify this point.

Both 69901A>C and 66292C>G SNPs are in intron regions, while 47686C>T is a synonymous variation and, as such, is probably not functional. These SNPs are considered preferable as genetic markers. Human *XDH* is located on chromosome 2 at p23.1. Recently, Angius *et al.* reported strong evidence that a 0.54-cM region of chromosome 2 (2p 26.5–27.1) harbors a locus-affecting risk of hypertension in an isolated Sardinian population (*45*). In addition, a number of regions of chromosome 2 (57–59, 86, 103, and 96–115 cM) have been found likely to harbor blood-pressure–modifying loci (*45–48*). More importantly, our group recently reported some hypertension-susceptibility genes at 2p24–p25 and a positive relationship between hypertension and SNPs of the Na^+/Ca^{2+} exchanger 1 gene, which is located at 2p22–p23, in a general Japanese population (*49, 50*). Expanded genotyping and a detailed cross-study of candidate genes are necessary.

In summary, in human *XDH*, we found three SNPs, 47686C>T, 67873A>C, and 69901A>C, that are significantly associated with hypertension. Another SNP, 66292C>G, was significantly associated with CKD, and 69901A>C also showed a positive relation to carotid atherosclerosis in hypertensive patients. These SNPs may be independent risk factors for hypertension or CKD and carotid atherosclerosis in hypertensive patients. There was a limitation in this study owing to its cross-sectional design. Prospective studies investigating the relationships between these SNPs and the development of hypertension, CKD, and atherosclerosis over a long term are necessary. These gene polymorphisms in *XDH* may be useful for predicting and preventing hypertension and its complications in future individualized treatment.

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References

1. Rutherford PA: Genetic influences in human hypertension. *J Hypertens* 2003; **21**: 19–22.
2. Andersson OK, Almgren T, Persson B, *et al*: Survival in treated hypertension: follow up study after two decades. *BMJ* 1998; **317**: 167–171.
3. Halushka MK, Fan JB, Bentley K, *et al*: Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. *Nat Genet* 1999; **22**: 239–247.
4. Smithies O, Maeda N: Gene targeting approaches to complex genetic diseases: atherosclerosis and essential hypertension. *Proc Natl Acad Sci U S A* 1995; **92**: 5266–5272.
5. Berry CE, Hare JM: Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J Physiol* 2004; **555**: 589–606.
6. Johnson RJ, Feig DI, Herrera-Acosta J, *et al*: Resurrection of uric acid as a causal risk factor in essential hypertension. *Hypertension* 2005; **45**: 18–20.
7. Niskanen LK, Laaksonen DE, Nyyssonen K, *et al*: Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. *Arch Intern Med* 2004; **164**: 1546–1551.
8. Freedman DS, Williamson DF, Gunter EW, *et al*: Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. *Am J Epidemiol* 1995; **141**: 637–644.
9. Bengtsson C, Lapidus L, Stendahl C, *et al*: Hyperuricaemia and risk of cardiovascular disease and overall death. A 12-year follow-up of participants in the population study of women in Gothenburg, Sweden. *Acta Med Scand* 1988; **224**: 549–555.
10. Klein R, Klein BE, Cornoni JC, *et al*: Serum uric acid. Its relationship to coronary heart disease risk factors and cardiovascular disease, Evans County, Georgia. *Arch Intern Med* 1973; **132**: 401–410.
11. Verdecchia P, Schillaci G, Reboldi G, *et al*: Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study. *Hypertension* 2000; **36**: 1072–1078.
12. Alderman MH, Cohen H, Madhavan S, *et al*: Serum uric acid and cardiovascular events in successfully treated hypertensive patients. *Hypertension* 1999; **34**: 144–150.
13. Iseki K, Oshiro S, Tozawa M, *et al*: Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. *Hypertens Res* 2001; **24**: 691–697.
14. Tomita M, Mizuno S, Yamanaka H, *et al*: Does hyperuricemia affect mortality? A prospective cohort study of Japanese male workers. *J Epidemiol* 2000; **10**: 403–409.
15. Yu TF, Berger L, Dorph DJ, *et al*: Renal function in gout. V. Factors influencing the renal hemodynamics. *Am J Med* 1979; **67**: 766–771.
16. Cai H, Harrison DG: Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000; **87**: 840–844.
17. Chabrashvili T, Tojo A, Onozato ML, *et al*: Expression and cellular localization of classic NADPH oxidase subunits in the spontaneously hypertensive rat kidney. *Hypertension* 2002; **39**: 269–274.
18. Beswick RA, Zhang H, Marable D, *et al*: Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. *Hypertension* 2001; **37**: 781–786.
19. Nishiyama A, Kobori H, Fukui T, *et al*: Role of angiotensin II and reactive oxygen species in cyclosporine A–dependent hypertension. *Hypertension* 2003; **42**: 754–760.
20. Meng S, Cason GW, Gannon AW, *et al*: Oxidative stress in Dahl salt-sensitive hypertension. *Hypertension* 2003; **41**: 1346–1352.
21. Wallwork CJ, Parks DA, Schmid-Schonbein GW: Xanthine oxidase activity in the dexamethasone-induced hypertensive rat. *Microvasc Res* 2003; **66**: 30–37.
22. Laakso J, Mervaala E, Himberg JJ, *et al*: Increased kidney xanthine oxidoreductase activity in salt-induced experimental hypertension. *Hypertension* 1998; **32**: 902–906.
23. Laakso JT, Teravainen TL, Martelin E, *et al*: Renal xanthine oxidoreductase activity during development of hypertension in spontaneously hypertensive rats. *J Hypertens* 2004; **22**: 1333–1340.
24. Landmesser U, Spickermann S, Dikalov S, *et al*: Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation* 2002; **106**: 3073–3078.
25. Lenda DM, Boegehold MA: Effect of a high-salt diet on oxidant enzyme activity in skeletal muscle microcirculation. *Am J Physiol Heart Circ Physiol* 2002; **282**: H395–H402.
26. Ulker S, McMaster D, McKeown PP, *et al*: Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. *Cardiovasc Res* 2003; **59**: 488–500.
27. Minoshima S, Wang Y, Ichida K, *et al*: Mapping of the gene for human xanthine dehydrogenase (oxidase) (XDH) to band p23 of chromosome 2. *Cytogenet Cell Genet* 1995; **68**: 52–53.
28. Kamide K, Takiuchi S, Tanaka C, *et al*: Three novel missense mutations of WNK4, a kinase mutated in inherited hypertension, in Japanese hypertensives: implication of clinical phenotypes. *Am J Hypertens* 2004; **17**: 446–449.
29. Kamide K, Tanaka C, Takiuchi S, *et al*: Six missense mutations of the epithelial sodium channel β and γ subunits in Japanese hypertensives. *Hypertens Res* 2004; **27**: 333–338.
30. Miwa Y, Takiuchi S, Kamide K, *et al*: Insertion/deletion polymorphism in clusterin gene influences serum lipid levels and carotid intima-media thickness in hypertensive Japanese females. *Biochem Biophys Res Commun* 2005; **331**: 1587–1593.
31. Takiuchi S, Mannami T, Miyata T, *et al*: Identification of 21 single nucleotide polymorphisms in human hepatocyte

growth factor gene and association with blood pressure and carotid atherosclerosis in the Japanese population. *Atherosclerosis* 2004; **173**: 301–307.
32. Yasuda H, Kamide K, Takiuchi S, *et al*: Association of single nucleotide polymorphisms in endothelin family genes with the progression of atherosclerosis in patients with essential hypertension. *J Hum Hypertens* 2007; **21**: 883–892.
33. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31–41.
34. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1–S266.
35. Tanaka C, Kamide K, Takiuchi S, *et al*: An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; **26**: 301–306.
36. Okuda T, Fujioka Y, Kamide K, *et al*: Verification of 525 coding SNPs in 179 hypertension candidate genes in the Japanese population: identification of 159 SNPs in 93 genes. *J Hum Genet* 2002; **47**: 387–394.
37. Mannami T, Baba S, Ogata J: Potential of carotid enlargement as a useful indicator affected by high blood pressure in a large general population of a Japanese city: the Suita study. *Stroke* 2000; **31**: 2958–2965.
38. Eriksen BO, Ingebretsen OC: The progression of chronic kidney disease: a 10-year population-based study of the effects of gender and age. *Kidney Int* 2006; **69**: 375–382.
39. Gelber RP, Kurth T, Kausz AT, *et al*: Association between body mass index and CKD in apparently healthy men. *Am J Kidney Dis* 2005; **46**: 871–880.
40. Agarwal R, Andersen MJ: Prognostic importance of clinic and home blood pressure recordings in patients with chronic kidney disease. *Kidney Int* 2006; **69**: 406–411.
41. Iseki K: Factors influencing the development of end-stage renal disease. *Clin Exp Nephrol* 2005; **9**: 5–14.
42. Li H, Samouilov A, Liu X, *et al*: Characterization of the magnitude and kinetics of xanthine oxidase–catalyzed nitrate reduction: evaluation of its role in nitrite and nitric oxide generation in anoxic tissues. *Biochemistry* 2003; **42**: 1150–1159.
43. Zhang Z, Blake DR, Stevens CR, *et al*: A reappraisal of xanthine dehydrogenase and oxidase in hypoxic reperfusion injury: the role of NADH as an electron donor. *Free Radic Res* 1998; **28**: 151–164.
44. Xu P, Huecksteadt TP, Harrison R, *et al*: Molecular cloning, tissue expression of human xanthine dehydrogenase. *Biochem Biophys Res Commun* 1994; **199**: 998–1004.
45. Angius A, Petretto E, Maestrale GB, *et al*: A new essential hypertension susceptibility locus on chromosome 2p24–p25, detected by genomewide search. *Am J Hum Genet* 2002; **71**: 893–905.
46. Krushkal J, Ferrell R, Mockrin SC, *et al*: Genome-wide linkage analyses of systolic blood pressure using highly discordant siblings. *Circulation* 1999; **99**: 1407–1410.
47. Rice T, Rankinen T, Province MA, *et al*: Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. *Circulation* 2000; **102**: 1956–1963.
48. Atwood LD, Samollow PB, Hixson JE, *et al*: Genome-wide linkage analysis of blood pressure in Mexican Americans. *Genet Epidemiol* 2001; **20**: 373–382.
49. Kamide K, Kokubo Y, Yang J, *et al*: Hypertension susceptibility genes on chromosome 2p24–p25 in a general Japanese population. *J Hypertens* 2005; **23**: 955–960.
50. Kokubo Y, Inamoto N, Tomoike H, *et al*: Association of genetic polymorphisms of sodium-calcium exchanger 1, NCX1, with hypertension in a Japanese general population. *Hypertens Res* 2004; **27**: 697–702.

Original Article

Plasma Adiponectin Is Associated with Plasma Brain Natriuretic Peptide and Cardiac Function in Healthy Subjects

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The aim of this study was to evaluate the relationship between the plasma adiponectin level, plasma brain natriuretic peptide (BNP) level, and cardiac function in healthy subjects. We obtained clinical data and performed blood tests, including measurement of the plasma adiponectin and BNP levels, in 1,538 healthy persons from Arita-cho, a rural area of Japan. Six hundred and eight subjects also underwent echocardiography. There was a significant positive correlation between their plasma BNP and adiponectin levels in simple regression analysis (standardized regression coefficient [β]=0.34). Multivariate regression analysis revealed that the plasma adiponectin level was independently associated with the plasma BNP level (β=0.12), as well as with the age (β=–0.26), male gender (β=–0.16), waist circumference (β=–0.16), and the plasma levels of high-density lipoprotein cholesterol (β=0.13), triglycerides (β=–0.16), aspartate aminotransferase (β=0.08), γ-glutamyl transpeptidase (β=–0.10), uric acid (β=–0.07), and creatinine (β=0.08). We also found a link between plasma adiponectin and the left atrial diameter index (β=0.08) or left ventricular diameter index (β=0.11), even after adjustment for age, sex, and body mass index. The plasma adiponectin level increased along with an increase of plasma BNP in healthy subjects independently of other confounding factors, demonstrating that adiponectin reflects cardiac function. (*Hypertens Res* 2008; 31: 825–831)

***Key Words*: adiponectin, brain natriuretic peptide, population based study, echocardiography**

Introduction

Adiponectin is one of the circulating adipocytokines, and it plays a role in regulating insulin sensitivity, lipid metabolism, and systemic inflammation (*1*). The plasma adiponectin level is decreased in persons with obesity, type 2 diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome and ischemic heart disease (*1–5*). We and others have previously reported that the severity of cardiac hypertrophy and subsequent heart failure is worsened in adiponectin knock-out mice subjected to pressure overload, suggesting that adiponectin may have a cardiovascular protective effect in patients with congestive heart failure (CHF) (*6–8*). Recent studies have indicated that the plasma adiponectin level is increased in patients with CHF, but the mechanism through which adiponectin production from adipose tissue is stimulated by CHF has not been elucidated (*9, 10*). The plasma brain natriuretic

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peptide (BNP) level has been reported to show a positive correlation with the plasma adiponectin level in patients with CHF or coronary heart disease (*9–12*). BNP secretion by the ventricular myocardium reflects the elevation of intracardiac pressure, and the circulating BNP level is related to the prognosis of CHF (*13, 14*). Even among apparently healthy subjects, relatively lower BNP levels predict a lower incidence of cardiovascular events, so BNP may reflect subtle hemodynamic changes in "healthy" persons (*15*). However, the relationship between plasma levels of BNP and adiponectin in healthy subjects has not yet been examined, and there has been no investigation of the relationship between the plasma adiponectin level and cardiac function.

To elucidate the factors predicting the plasma adiponectin level and to investigate the relationship between adiponectin and cardiac function, we performed a cross-sectional study of 1,538 healthy subjects without CHF from Arita-cho, a rural area in Japan. We investigated the predictors of adiponectin, including BNP, and also assessed the relationship between the plasma adiponectin level and echocardiographic parameters of cardiac function.

Methods

The study was conducted in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the National Cardiovascular Center and that of Arita-cho. Written informed consent was obtained from each subject before participation in this study in accordance with approved institutional protocols.

Study Population

We studied 1,538 persons from Arita-cho who had no clinical evidence of overt CHF. Laboratory tests, including plasma adiponectin and BNP levels, were performed in all subjects and 608 of them also underwent echocardiography.

Assays

The plasma concentration of adiponectin was measured using a specific immunoradiometric assay (*2*). The plasma concentration of BNP was measured with a specific immunoradiometric assay for human BNP using a commercial kit (Shionoria; Shionogi Co., Ltd., Osaka, Japan) (*14*). The insulin resistance index was determined by the homeostasis model assessment of insulin resistance (HOMA-R) method (*16*).

Echocardiography

The left atrial diameter, the left ventricular end-diastolic and end-systolic dimensions, the left ventricular fractional shortening, and the left ventricular septal and posterior wall thickness were obtained by M-mode echocardiography. The left ventricular mean wall thickness was calculated by averaging the left ventricular septal and posterior wall thickness. All echocardiograms were read by physicians from the National Cardiovascular Center who were blinded to any clinical information about the subjects. Indexed values were calculated by dividing the results by the body surface area, which was calculated according to Mosteller (*17*). The left ventricular mass was calculated using parameters measured by M-mode echocardiography (*18*). Mitral inflow was assessed in the api-

Table 1. Baseline Characteristics of 1,538 Subjects

Demographic data	
Age* (years)	62±15
Female/male (%)	63/37
Body mass index* (kg/m^2)	22±3
Waist circumference* (cm)	83±9
Concomitant diseases, % (*n*)	
Ischemic heart disease	3.4 (53)
Chronic atrial fibrillation	0.2 (3)
Hypertension	27 (408)
Diabetes mellitus	5.7 (88)
Hyperlipidemia	10 (160)
Hormonal status	
Adiponectin† (μg/mL)	10.6 (7.6–15.2)
BNP† (pg/mL)	18.0 (9.4–34.0)
Glucose metabolism	
Fasting blood glucose† (mg/dL)	83 (78–90)
Fasting insulin† (μU/mL)	4 (3–6)‡
HOMA-R†	0.9 (0.6–1.3)‡
Hemoglobin A1c* (%)	5.3±0.8
Lipids	
Total cholesterol* (mg/dL)	194±31
HDL cholesterol* (mg/dL)	55±12
Triglycerides† (mg/dL)	88 (63–122)
LDL cholesterol* (mg/dL)	119±29
Other parameters	
Hemoglobin* (g/dL)	13.4±1.5
AST† (IU/L)	22 (19–26)
ALT† (IU/L)	18 (14–23)
γ-GTP† (IU/L)	22 (16–37)
Uric acid* (mg/dL)	4.9±1.4
Creatinine* (mg/dL)	0.9±0.2
Blood pressure	
SBP* (mmHg)	128±20
DBP* (mmHg)	77±11
MBP* (mmHg)	94±13

BNP, brain natriuretic peptide; HOMA-R, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure. *Values are shown as the mean±SD. †Values are shown as the median (interquartile range). ‡n=800.

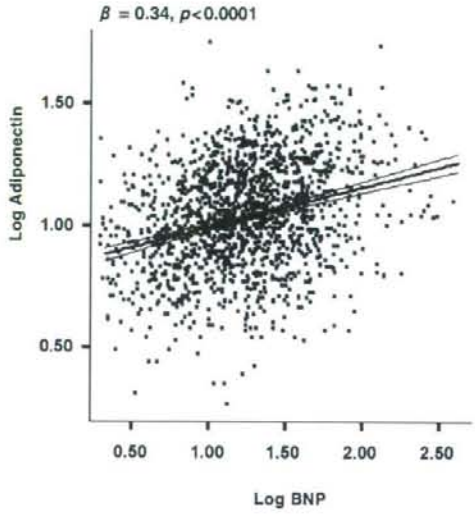

Fig. 1. *Scatter plot of the association between log-transformed plasma adiponectin and plasma BNP levels in apparently healthy subjects from Arita-cho. Lines indicate the regression line (thick line) and 95% confidence interval (thin lines). BNP, brain natriuretic peptide; β, standardized regression coefficient.*

cal four-chamber view using pulsed-wave Doppler echocardiography, with the Doppler beam aligned parallel to the direction of the flow and the sample volume set at the leaflet tips. From the mitral inflow profile, the peak velocity of early diastolic transmitral flow, the peak velocity of late diastolic transmitral flow, and the early diastolic transmitral flow deceleration time were measured (*19*).

Statistical Analysis

Numerical data are reported as the mean±SD or the median and interquartile range, as appropriate. Data on the levels of adiponectin, BNP, fasting insulin, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP) were logarithmically transformed for linear regression analysis. Multivariate analysis was done to examine the correlations of log-transformed adiponectin levels with the variables that were associated with adiponectin ($p<0.10$) according to simple regression analysis. The final model always included the following potentially confounding variables irrespective of the results of univariate analysis; age, sex, body mass index (BMI), ischemic heart disease, and atrial fibrillation. Fasting insulin levels and HOMA-R were not included in the final model because of missing data for a significant number of subjects.

Echocardiographic parameters were assessed for their relationship with adiponectin (log-transformed) by univariate analysis or analysis adjusted for age, gender, and BMI. Interaction effects of gender, age (65 years or older), ischemic heart disease, hypertension, diabetes mellitus, and hyperlipidemia on the relation between plasma adiponectin and BNP levels were examined with analysis of covariance. A probability (p) value <0.05 was accepted as indicating statistical significance unless otherwise mentioned. A software package (SPSS 10.0.5J; SPSS, Chicago, USA) was used for statistical analysis.

Results

The profile of the subjects is shown in Table 1. There was a significant positive correlation between their plasma BNP and adiponectin levels (Fig. 1). This relationship was not influenced by covariates such as gender, age (65 years or older), ischemic heart disease, hypertension, diabetes mellitus, or hyperlipidemia.

Predictors of the Adiponectin Level

Table 2 shows the factors from among clinical, laboratory, and hemodynamic parameters that showed correlations with the plasma adiponectin levels. Univariate analysis revealed that male sex and diabetes mellitus were associated with lower adiponectin levels. The BMI, waist circumference, fasting blood glucose, fasting insulin, HOMA-R, triglycerides, hemoglobin, ALT, γ-GTP, uric acid, creatinine, and blood pressure were all negatively correlated with the plasma adiponectin level, whereas age, plasma BNP, and plasma high-density lipoprotein (HDL)-cholesterol were positively correlated with adiponectin. Among these factors, it was found that male sex, waist circumference (but not BMI), and the plasma levels of triglycerides, AST, γ-GTP, and uric acid were negatively correlated with adiponectin by multivariate analysis, while there were positive correlations for age and the plasma levels of BNP, HDL-cholesterol, and creatinine. In the present cross-sectional study, sex was most strongly associated with the plasma adiponectin level, followed by age, according to the standardized regression coefficients (β). The plasma BNP level showed as strong an association with the plasma adiponectin level as did waist circumference, HDL-cholesterol, and triglycerides.

Echocardiographic Parameters

Since the plasma adiponectin level is elevated in patients with heart failure, we examined whether adiponectin was related to cardiac function. The baseline echocardiographic parameters are displayed in Table 3.

According to univariate analysis (Table 4), several echocardiographic parameters were related to the plasma adiponectin level, including the left atrial diameter index, left ventricular

Table 2. Factors Correlated with Plasma Adiponectin by Regression Analysis

	Univariate		Multivariate	
	Standardized coefficient	*p* value	Standardized coefficient	*p* value
r^2 of the test			0.40	<0.0001
Age	0.26	<0.0001	0.22	<0.0001
Male sex	−0.38	<0.0001	−0.26	<0.0001
Body mass index	−0.27	<0.0001	−0.05	0.2247
Waist circumference	−0.29	<0.0001	−0.16	0.0001
Ischemic heart disease	0.01	0.6838		
Hypertension	<0.01	0.9972		
Diabetes	−0.06	0.0230	<0.01	0.9911
Hyperlipidemia	<0.01	0.8449		
Log BNP	0.34	<0.0001	0.12	<0.0001
Fasting blood glucose	−0.08	0.0440	−0.04	0.0716
Log fasting insulin*	−0.22	<0.0001		
HOMA-R*	−0.15	<0.0001		
Hemoglobin A1c	−0.04	0.3020		
Total cholesterol	0.02	0.5580		
HDL-cholesterol	0.26	<0.0001	0.13	<0.0001
Log triglycerides	−0.29	<0.0001	−0.16	<0.0001
LDL-cholesterol	0.05	0.2140		
Hemoglobin	−0.42	<0.0001	−0.05	0.0811
Log AST	−0.07	0.0810	0.08	0.0206
Log ALT	−0.29	<0.0001	−0.03	0.3611
Log γ-GTP	−0.25	<0.0001	−0.10	0.0004
Uric acid	−0.29	<0.0001	−0.07	0.0114
Creatinine	−0.16	<0.0001	0.08	0.0050
SBP	−0.01	0.7480		
DBP	−0.08	0.0470		
MBP	−0.08	0.0029	0.04	0.1077

BNP, brain natriuretic peptide; HOMA-R, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure. *Log fasting insulin and HOMA-R were not included in the multivariate model, because insulin was not measured in a considerable number of subjects.

Table 3. Echocardiographic Data Obtained in 608 Subjects

Left atrial diameter (mm)	37 (33–41)
Left atrial diameter index (mm/m^2)	24 (22–27)
Left ventricular end-diastolic diameter (mm)	46 (43–50)
Left ventricular end-diastolic diameter index (mm/m^2)	30 (28–33)
Fractional shortening (%)	40 (35–45)
Mean wall thickness (mm)	9 (8–10)
Left ventricular mass (g)	140 (114–166)
Left ventricular mass index (g/m^2)	90 (76–106)
E wave velocity (cm/s)	63 (53–74)
A wave velocity (cm/s)	77 (64–89)
E/A ratio	0.82 (0.69–0.99)
Deceleration time (ms)	217 (192–258)

Values are shown as the median (interquartile range). E, early diastolic transmitral flow; A, late diastolic transmitral flow.

end-diastolic diameter index, the fractional shortening, the mean wall thickness, the left ventricular mass index, the late diastolic transmitral flow velocity, and the early/late diastolic transmitral flow velocity ratio. After adjustment for age, sex and BMI, however, only the left atrial diameter index and the left ventricular end-diastolic diameter index were positively correlated with the circulating adiponectin level.

Discussion

The present study demonstrated that the plasma adiponectin level was positively correlated with the plasma BNP level in healthy subjects along with known predictors such as the age, BMI, waist circumference, HDL-cholesterol, triglycerides, liver function parameters, uric acid, and renal function parameters. The plasma BNP level had the same influence on adiponectin as that of the waist circumference, HDL-cholesterol, and triglycerides. We also demonstrated that the plasma adi-

Table 4. Factors Correlated with the Plasma Level of Adiponectin in Regression Analysis

	Univariate		Adjusted for age, sex, and BMI	
	Standardized coefficient	*p* value	Standardized coefficient	*p* value
Left atrial diameter	−0.09	0.0326	0.05	0.2528
Left atrial diameter index	0.24	<0.0001	0.08	0.0312
Left ventricular end-diastolic diameter	−0.16	0.0001	0.06	0.1035
Left ventricular end-diastolic diameter index	0.30	<0.0001	0.11	0.0045
Fractional shortening	0.09	0.0354	−0.03	0.4557
Mean wall thickness	−0.09	0.0266	−0.02	0.6545
Left ventricular mass	−0.17	<0.0001	0.03	0.4725
Left ventricular mass index	0.02	0.6362	0.05	0.2028
E wave velocity	−0.01	0.8528	<0.01	0.9886
A wave velocity	0.15	0.0003	−0.01	0.8500
E/A ratio	−0.13	0.0013	0.02	0.6726
Deceleration time	0.06	0.1439	0.01	0.7182

BMI, body mass index; E, early diastolic transmitral flow; A, late diastolic transmitral flow.

ponectin level reflects cardiac function to some extent, even in apparently healthy subjects.

Link between Plasma Adiponectin and BNP

The plasma adiponectin level is reported to be increased and to show a positive correlation with plasma BNP in CHF patients (*9*, *10*). It has been suggested that cardiac cachexia due to the progression of heart failure leads to an increase of plasma adiponectin levels (*7*). However, this study revealed that "healthy" subjects without cardiac cachexia also demonstrated a positive correlation between plasma adiponectin and BNP levels, suggesting that cardiac cachexia itself is unlikely to be the reason for the positive relationship between plasma adiponectin and BNP levels.

In obese patients, BNP levels are lower, due to increased expression of the natriuretic clearance receptor in adipose tissue or some other unknown factor (*20*). Because adiponectin levels are also lower in obese patients, one may argue that the relationship between the plasma adiponectin and BNP is confounded by obesity (*2*). Renal dysfunction is known to increase the levels of both adiponectin and BNP (*21*, *22*). However, the influence of obesity and renal dysfunction cannot explain the adiponectin-BNP relationship that was revealed by the present observational study, which found a tight relationship between plasma adiponectin and BNP independently of these potentially confounding factors.

The final possibility is that BNP directly increases adiponectin production. Indeed, we have preliminary data showing that BNP directly increases adiponectin mRNA expression in cultured 3T3-L1 adipocytes (data not shown). Wang *et al.* reported that low BNP was associated with metabolic risk factors in ambulatory individuals (*23*), and that these risk factors could be mediated by adiponectin, which was consistent with a tight linkage between adiponectin and BNP levels. Higher levels of adiponectin could constitute a risk for CHF, and at the same time could be a negative risk for metabolic syndrome through their interaction with BNP; lower levels of BNP could constitute a negative risk for CHF and at the same a risk for metabolic syndrome through their interaction with adiponectin.

Adiponectin and Cardiac Function

Wang *et al.* reported that the extent of the increase in plasma BNP levels could predict cardiovascular mortality in subjects without CHF, indicating that subtle stress on the heart can be detected by monitoring BNP and this stress leads to cardiovascular events (*15*).

We demonstrated that some conventional echocardiographic parameters (left atrial and left ventricular diameter indexes) showed a relationship with adiponectin similar to the relation of BNP to adiponectin. Previous studies of the relationship between adiponectin and BNP have also found no obvious cardiac dysfunction in their subjects, but they did not examine echocardiographic parameters (*11*, *12*).

Cardiac dimensions are not direct indexes of cardiac function. However, it is also the case that left ventricular dimensions are tightly related to cardiac function. As cardiac function is depressed, end-diastolic or systolic ventricular dimension increases, and vise versa. Even subjects with high-normal blood pressure suffer from some degree of left ventricular remodeling (*24*). The left atrial dimension, a predictor of atrial fibrillation, reflects blood pressure levels and metabolic abnormalities in ambulatory settings (*25*). Plasma adiponectin may increase to counteract these latent cardiac remodeling effects.

How does hemodynamics or structural change regulate adiponectin production? One possibility is that BNP directly causes an increase of adiponectin production, as mentioned above. Alternatively, there is a possibility that adiponectin is produced in the heart when it is placed under stress. It has

been reported that adiponectin is synthesized and secreted by isolated murine and human cardiomyocytes (*26*). However, the production of adiponectin by adipose tissue is greater than that by cardiac tissue (in part because the amount of adipose tissue is greater), suggesting that most of the circulating adiponectin is derived from adipose tissue.

Cardioprotective Role of BNP and Adiponectin

BNP is known to have a protective effect against cardiovascular injury *via* the cyclic GMP-G kinase pathway, and an increase of BNP is believed to improve the severity of heart failure (*14*, *27*, *28*). BNP may exert a cardioprotective effect on subclinical heart disease (*15*).

We and another group have recently reported that cardiac hypertrophy and subsequent heart failure are accelerated by aortic banding in adiponectin knock-out mice (*6*, *8*). Cardiac hypertrophy is believed to be a major risk factor for cardiovascular death, suggesting that adiponectin release is increased by stresses acting on the heart even in healthy subjects for prevention of cardiac injury like BNP (*29*). Adiponectin may decrease the onset or the progression of CHF. Pioglitazone has been reported to increase adiponectin production *via* stimulation of peroxisome proliferator–activated receptor γ and it also reduces cardiac hypertrophy in mice with aortic banding (*30*). Thus, adiponectin may be a target for prevention of overt CHF.

Clinical Implications

The positive correlation between plasma adiponectin and BNP levels in apparently healthy individuals in our study suggests that adiponectin can predict a worsening of prognosis or morbidity of heart failure even in healthy subjects. In addition, we need to consider whether latent heart failure and BNP levels should be taken into account when we examine subjects with higher adiponectin levels in clinical settings. Further prospective investigations are therefore warranted to determine whether high adiponectin levels are related to worse prognosis or future morbidity of heart failure even in healthy subjects.

Limitations

The present large-scale cross-sectional study showed a close relationship between adiponectin and BNP. However, the nature of a cross-sectional study means that we could not assess the prognostic impact of adiponectin in our healthy subjects. In CHF patients, high adiponectin levels are related to a worse prognosis, so longitudinal studies are needed to elucidate the impact of adiponectin on the prognosis in apparently healthy persons (*9*, *10*).

We used self-administered questionnaires to investigate the rates of risk factors and the current state of treatment. The percentages of subjects taking medication were 81%, 61%, and 68% for the patients with hypertension, diabetes mellitus, and hyperlipidemia, respectively. There were no significant differences in adiponectin and BNP values between those taking and those not taking medications. Thus medications do not seem to have influenced the results significantly.

Conclusions

The plasma adiponectin level increased along with an increase of the plasma BNP level in apparently healthy subjects, suggesting that plasma adiponectin reflects some aspects of cardiac function.

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References

1. Santaniemi M, Kesaniemi YA, Ukkola O: Low plasma adiponectin concentration is an indicator of the metabolic syndrome. *Eur J Endocrinol* 2006; **155**: 745–750.
2. Arita Y, Kihara S, Ouchi N, *et al*: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79–83.
3. Hotta K, Funahashi T, Arita Y, *et al*: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595–1599.
4. Okamoto Y, Kihara S, Funahashi T, Matsuzawa Y, Libby P: Adiponectin: a key adipocytokine in metabolic syndrome. *Clin Sci (Lond)* 2006; **110**: 267–278.
5. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB: Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004; **291**: 1730–1737.
6. Liao Y, Takashima S, Maeda N, *et al*: Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovasc Res* 2005; **67**: 705–713.
7. Ouchi N, Shibata R, Walsh K: Cardioprotection by adiponectin. *Trends Cardiovasc Med* 2006; **16**: 141–146.
8. Shibata R, Ouchi N, Ito M, *et al*: Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med* 2004; **10**: 1384–1389.
9. George J, Patal S, Wexler D, *et al*: Circulating adiponectin concentrations in patients with congestive heart failure. *Heart* 2006; **92**: 1420–1424.
10. Kistorp C, Faber J, Galatius S, *et al*: Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation* 2005; **112**: 1756–1762.
11. Pilz S, Mangge H, Wellnitz B, *et al*: Adiponectin and mortality in patients undergoing coronary angiography. *J Clin*

Endocrinol Metab 2006; **91**: 4277–4286.
12. von Eynatten M, Hamann A, Twardella D, Nawroth PP, Brenner H, Rothenbacher D: Relationship of adiponectin with markers of systemic inflammation, atherogenic dyslipidemia, and heart failure in patients with coronary heart disease. *Clin Chem* 2006; **52**: 853–859.
13. Mukoyama M, Nakao K, Hosoda K, *et al*: Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest* 1991; **87**: 1402–1412.
14. Tsutamoto T, Wada A, Maeda K, *et al*: Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. *Circulation* 1997; **96**: 509–516.
15. Wang TJ, Larson MG, Levy D, *et al*: Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* 2004; **350**: 655–663.
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
17. Mosteller RD: Simplified calculation of body-surface area. *N Engl J Med* 1987; **317**: 1098.
18. Devereux RB, Alonso DR, Lutas EM, *et al*: Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; **57**: 450–458.
19. Kitabatake A, Inoue M, Asao M, *et al*: Transmitral blood flow reflecting diastolic behavior of the left ventricle in health and disease—a study by pulsed Doppler technique. *Jpn Circ J* 1982; **46**: 92–102.
20. Wang TJ, Larson MG, Levy D, *et al*: Impact of obesity on plasma natriuretic peptide levels. *Circulation* 2004; **109**: 594–600.
21. Tsutamoto T, Wada A, Sakai H, *et al*: Relationship between renal function and plasma brain natriuretic peptide in patients with heart failure. *J Am Coll Cardiol* 2006; **47**: 582–586.
22. Zoccali C, Mallamaci F, Tripepi G, *et al*: Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 2002; **13**: 134–141.
23. Wang TJ, Larson MG, Keyes MJ, Levy D, Benjamin EJ, Vasan RS: Association of plasma natriuretic peptide levels with metabolic risk factors in ambulatory individuals. *Circulation* 2007; **115**: 1345–1353.
24. Kimura Y, Tomiyama H, Nishikawa E, *et al*: Characteristics of cardiovascular morphology and function in the high-normal subset of hypertension defined by JNC-VI recommendations. *Hypertens Res* 1999; **22**: 291–295.
25. Vaziri SM, Larson MG, Lauer MS, Benjamin EJ, Levy D: Influence of blood pressure on left atrial size. The Framingham Heart Study. *Hypertension* 1995; **25**: 1155–1160.
26. Pineiro R, Iglesias MJ, Gallego R, *et al*: Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Lett* 2005; **579**: 5163–5169.
27. D'Souza SP, Yellon DM, Martin C, *et al*: B-type natriuretic peptide limits infarct size in rat isolated hearts *via* KATP channel opening. *Am J Physiol Heart Circ Physiol* 2003; **284**: H1592–H1600.
28. Nishikimi T, Maeda N, Matsuoka H: The role of natriuretic peptides in cardioprotection. *Cardiovasc Res* 2006; **69**: 318–328.
29. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP: Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; **322**: 1561–1566.
30. Asakawa M, Takano H, Nagai T, *et al*: Peroxisome proliferator-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy *in vitro* and *in vivo*. *Circulation* 2002; **105**: 1240–1246.

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 *Alu* 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. *Hin*dIII restriction fragment length polymorphism (*12*) is one of the most widely studied variants of the Y chromosome. Australian men negative for the *Hin*dIII restriction site have been shown to have significantly higher DBP than *Hin*dIII positive men (*13*), whereas a study based on Polish and Scottish men reported an association between the presence of the *Hin*dIII restriction site and elevated levels of SBP and DBP (*14*). The presence of the *Hin*dIII restriction site was more prevalent among hypertensive than normotensive subjects (*15*). Men with *Hin*dIII(−) genotype had significantly higher levels of LDL and total cholesterol (TC) than those with *Hin*dIII(+) genotype (*16*). In contrast, no association was found between *Hin*dIII(+/−) 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 *Hin*dIII 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 *Hin*dIII 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≥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±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 3. Association between YAP Polymorphism and Cardiovascular Risk Factors

	YAP(−) (n=647)	YAP(+) (n=291)	Nominal p	Corrected p
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.
W/H 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*[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**[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**[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 [a]age, [b]age, BMI, ethanol consumption (g/d) and number of cigarettes per day, [c]age and BMI, [d]age-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 p	Corrected p
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.
W/H 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*[,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**[,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**[,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***[,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***[,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 [a]age, [b]ethanol consumption (g/d) and number of cigarettes per day, [c]age and BMI, [d]age-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 p	Corrected p
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.
W/H 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*[,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**[,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**[,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 [a]age, [b]ethanol consumption (g/d) and number of cigarettes per day, [c]age and BMI, [d]age-squared and BMI. See Table 3 for abbreviations.

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

	n (%)	HTN (%)	χ^1		HDL cholesterol (mg/dL)	ANOVA	
			Nominal *p*	Corrected *p*		Nominal *p**	Corrected *p**
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 *Hin*dIII polymorphism in the non-recombining region of the Y chromosome on BP and lipid levels have produced discrepant results. Since genotyping of the *Hin*dIII 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, *Hin*dIII negative men were taller than *Hin*dIII positive men: 174.7±6.6 cm *vs.* 172.8±7.7 cm, p=0.009 (*31*). Absence of the *Hin*dIII 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 *Hin*dIII 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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References

1. Keil JE, Sutherland SE, Knapp RG, Lackland DT, Gazes PC, Tyroler HA: Mortality rates and risk factors for coronary disease in black as compared with white men and women. *N Engl J Med* 1993; **329**: 73–78.
2. Levi F, Lucchini F, Negri E, La Vecchia C: Trends in mortality from cardiovascular and cerebrovascular diseases in Europe and other areas of the world. *Heart* 2002; **88**: 119–124.
3. Manuel DG, Leung M, Nguyen K, Tanuseputro P, Johansen H: Burden of cardiovascular disease in Canada. *Can J Cardiol* 2003; **19**: 997–1004.
4. Martiniuk AL, Lee CM, Lawes CM, *et al*: Hypertension: its prevalence and population-attributable fraction for mortality from cardiovascular disease in the Asia-Pacific region. *J Hypertens* 2007; **25**: 73–79.
5. Wolf-Maier K, Cooper RS, Banegas JR, *et al*: Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *JAMA* 2003; **289**: 2363–2369.
6. Musha H, Hayashi A, Kida K, *et al*: Gender difference in the level of high-density lipoprotein cholesterol in elderly Japanese patients with coronary artery disease. *Intern Med* 2006; **45**: 241–245.
7. Ishizaka N, Ishizaka Y, Toda E, *et al*: Association between obesity and chronic kidney disease in Japanese: differences in gender and hypertensive status? *Hypertens Res* 2007; **30**: 1059–1064.
8. Ely DL, Turner ME: Hypertension in the spontaneously hypertensive rat is linked to the Y chromosome. *Hypertension* 1990; **16**: 277–281.
9. Ely DL, Daneshvar H, Turner ME, Johnson ML, Salisbury RL: The hypertensive Y chromosome elevates blood pressure in F11 normotensive rats. *Hypertension* 1993; **21**: 1071–1075.
10. Ely D, Caplea A, Dunphy G, *et al*: Spontaneously hypertensive rat Y chromosome increases indexes of sympathetic nervous system activity. *Hypertension* 1997; **29**: 613–618.
11. Kren V, Qi N, Krenova D, *et al*: Y-chromosome transfer induces changes in blood pressure and blood lipids in SHR. *Hypertension* 2001; **37**: 1147–1152.
12. Santos FR, Pena SD, Tyler-Smith C: PCR haplotypes for the human Y chromosome based on alphoid satellite DNA variants and heteroduplex analysis. *Gene* 1995; **165**: 191–198.
13. Ellis JA, Stebbing M, Harrap SB: Association of the human Y chromosome with high blood pressure in the general population. *Hypertension* 2000; **36**: 731–733.
14. Charchar FJ, Tomaszewski M, Padmanabhan S, *et al*: The Y chromosome effect on blood pressure in two European populations. *Hypertension* 2002; **39**: 353–356.
15. Garcia EC, Gonzalez P, Castro MG, *et al*: Association between genetic variation in the Y chromosome and hypertension in myocardial infarction patients. *Am J Med Genet A* 2003; **122**: 234–237.
16. Charchar FJ, Tomaszewski M, Lacka B, *et al*: Association of the human Y chromosome with cholesterol levels in the general population. *Arterioscler Thromb Vasc Biol* 2004; **24**: 308–312.
17. Russo P, Venezia A, Lauria F, *et al*: *Hin*dIII(+/–) polymorphism of the Y chromosome, blood pressure, and serum lipids: no evidence of association in three white populations. *Am J Hypertens* 2006; **19**: 331–338.
18. Shankar RR, Charchar FJ, Eckert GJ, *et al*: Studies of an association in boys of blood pressure and the Y chromosome. *Am J Hypertens* 2007; **20**: 27–31.
19. Shoji M, Tsutaya S, Shimada J, Kojima K, Kasai T, Yasujima M: Lack of association between Y chromosome Alu insertion polymorphism and hypertension. *Hypertens Res* 2002; **25**: 1–3.
20. Hammer MF, Horai S: Y chromosomal DNA variation and the peopling of Japan. *Am J Hum Genet* 1995; **56**: 951–962.
21. Mannami T, Konishi M, Baba S, Nishi N, Terao A: Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. *Stroke* 1997; **28**: 518–525.
22. Iwai N, Katsuya T, Mannami T, *et al*: Association between *SAH*, an acyl-CoA synthetase gene, and hypertriglyceridemia, obesity, and hypertension. *Circulation* 2002; **105**: 41–47.
23. Shioji K, Kokubo Y, Mannami T, *et al*: Association between hypertension and the α-adducin, β1-adrenoreceptor, and G-protein β3 subunit genes in the Japanese population; the Suita study. *Hypertens Res* 2004; **27**: 31–37.
24. Kokubo Y, Iwai N, Tago N, *et al*: Association analysis between hypertension and *CYBA*, *CLCNKB*, and *KCNMB1* functional polymorphisms in the Japanese population—the

Suita Study. *Circ J* 2005; **69**: 138–142.
25. Iwai N, Kajimoto K, Kokubo Y, *et al*: Assessment of genetic effects of polymorphisms in the MCP-1 gene on serum MCP-1 levels and myocardial infarction in Japanese. *Circ J* 2006; **70**: 805–809.
26. Iwai N, Kajimoto K, Tomoike H, Takashima N: Polymorphism of *CYP11B2* determines salt sensitivity in Japanese. *Hypertension* 2007; **49**: 825–831.
27. Takashima N, Shioji K, Kokubo Y, *et al*: Validation of the association between the gene encoding proteasome subunit alpha type 6 and myocardial infarction in a Japanese population. *Circ J* 2007; **71**: 495–498.
28. Shinka T, Tomita K, Toda T, *et al*: Genetic variations on the Y chromosome in the Japanese population and implications for modern human Y chromosome lineage. *J Hum Genet* 1999; **44**: 240–245.
29. Underhill PA, Passarino G, Lin AA, *et al*: The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. *Ann Hum Genet* 2001; **65**: 43–62.
30. Jin HJ, Kwak KD, Hammer MF, *et al*: Y-chromosomal DNA haplogroups and their implications for the dual origins of the Koreans. *Hum Genet* 2003; **114**: 27–35.
31. Ellis JA, Stebbing M, Harrap SB: Significant population variation in adult male height associated with the Y chromosome and the aromatase gene. *J Clin Endocrinol Metab* 2001; **86**: 4147–4150.
32. Reckelhoff JF, Zhang H, Granger JP: Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension* 1998; **31**: 435–439.
33. Reckelhoff JF, Zhang H, Srivastava K, Granger JP: Gender differences in hypertension in spontaneously hypertensive rats: role of androgens and androgen receptor. *Hypertension* 1999; **34**: 920–923.

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Validation of the Association of Genetic Variants on Chromosome 9p21 and 1q41 With Myocardial Infarction in a Japanese Population

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Background Recent large-scale genome-wide association studies have identified several loci associated with the risk of coronary artery disease (CAD). The aim of the present study was to examine whether the previously reported CAD-associated single-nucleotide polymorphisms (SNPs) confer susceptibility to myocardial infarction (MI) in a study population of 2,475 controls and 589 cases of MI. The effect of the CAD-associated SNPs on cardiovascular risk factors in the control group was also investigated.

Methods and Results Significant associations were observed between 2 SNPs, rs1333049 on chromosome 9p21 and rs17465637 on chromosome 1q41, and MI, with odds ratios adjusted for age, sex, diabetes, hypertension and smoking habit of 1.47 (95% confidence interval (CI), 1.15–1.89; corrected p=0.006) and 1.45 (95%CI, 1.15–1.83; corrected p=0.006) for rs1333049 and rs17465637, respectively. None of the genotypes was associated with body mass index, plasma lipid profile, blood pressure, glucose, or hemoglobin A_{1c}. The genotypes also had no effect on the marker of inflammation (C-reactive protein) or atherosclerosis (mean and maximum carotid intima–media thickness).

Conclusions Although the underlying mechanisms are not clearly understood, the previously reported association between the 2 SNPs (rs1333049 and rs17465637) and MI was reproduced in this Japanese sample. (*Circ J* 2008; **72:** 1213–1217)

Key Words: Genetics; Myocardial infarction; Polymorphism

Based on recent large-scale genome-wide association studies, several loci associated with the risk of coronary artery disease (CAD) have been identified[1-3] among which the chromosome 9p21 has been the most consistent locus showing a strong association with CAD. A genome-wide scan conducted in Canadians (322 cases, 312 controls) and subsequent validation studies involving more than 2,326 cases and 10,427 controls found the genetic variations in the chromosome 9p21 region (rs10757274 and rs2383206) to be associated with CAD.[2] Similarly, the genome wide association analysis in an Icelandic population demonstrated the association between myocardial infarction (MI) and the single-nucleotide polymorphisms (SNPs) on the chromosome 9p21 locus (rs1333040, rs2383207, and rs10116277).[1] Combined analysis of the initial Icelandic and 4 replication groups from Iceland and the United States revealed rs10757278 on chromosome 9p21 as the most significant SNP, with the population attributable risk for MI being estimated as 21% in general.[1] In the analysis of the Welcome Trust Case Control Consortium (WTCCC) study,[3] genotyping was carried out in 1,926 CAD cases and 2,938 controls using the GeneChip Human Mapping 500K array (Affymetrix). Nine chromosomal regions were found to be significantly associated with CAD, 3 of which remained significant after testing for the association with MI in the German MI Family Study composed of 875 cases with MI and 1,644 controls[4] with the chromosome 9p21.3 locus showing the strongest association in both studies. Combined analysis of the WTCCC study and the German MI Family Study revealed an additional 4 loci associated with the increased risk of CAD.

Despite the different SNPs investigated from study to study, associations between the SNPs on chromosome 9p21 and the risk of CAD or MI have been consistently reported in case–control studies from different countries including South Korea[5] Italy[6,7] Germany, Sweden, and the United Kingdom[8] The underlying mechanisms for the association between these susceptibility loci identified through genome-wide scanning and the risk of CAD/MI remain to be elucidated. There has been no evidence of an association between the risk alleles of SNPs on chromosome 9p and conventional risk factors such as lipid parameters or blood pressure levels[2,6] As stressed by the National Cancer Institute-National Human Genome Research Institute (NCI-NHGRI) Working Group on Replication in Association Studies[9] validation of the postulated gene–disease associations in a population different from that of the initial study is of great value. Thus, the aim of the present study was to examine whether the previously reported CAD-associated SNPs, including those located on chromosome 9p21, confer

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