

Association between Angiotensin II Type 2 Receptor Gene A/C3123 Polymorphism and High-Density Lipoprotein Cholesterol with Hypertension in Asymptomatic Women

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Key Words

Renin-angiotensin system · Single-nucleotide polymorphisms · Cholesterol metabolism · Atherosclerosis

Abstract

Objective: The present study investigated the association between the angiotensin II type 2 receptor (AT2R) gene adenine/cytosine (A/C)-3123 polymorphism and cardiometabolic variables in subjects with and without hypertension.

Methods: Cardiometabolic variables, in addition to genotyping by an allele-specific DNA assay, were measured in 161 asymptomatic community-dwelling Japanese women (age range 30–83 years). They were divided into hypertensive (n = 82, age 50–81 years) and nonhypertensive (n = 79, age 30–83 years) subjects. **Results:** The A-allele carriers (n = 53) showed significantly lower high-density lipoprotein cholesterol (HDL-C) levels than the non-A-allele carriers (n = 26) among nonhypertensive subjects (1.45 ± 0.38 vs. 1.66 ± 0.33 mmol/l, $p = 0.02$). Even when multiple-adjusted analyses were performed, the HDL-C levels continued to differ significantly and independently of other variables, including the body mass index and insulin resistance index, between A-allele and non-A-allele carriers. However, this association was not observed among hypertensive subjects. **Conclu-**

sion: The present study demonstrated that A-allele carriers had significantly lower HDL-C levels than did non-A-allele carriers among nonhypertensive women, while this association was not observed among hypertensive women. This indicates that the A/C3123 polymorphism may be a marker associated with HDL metabolism by hypertension. This was a small study, so further research is warranted to confirm the observed association.

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Introduction

The renin-angiotensin system and its related single-nucleotide gene polymorphisms have been studied as a key to understanding the pathophysiology of cardiometabolic diseases, including hypertension, and to developing therapies for these diseases [1]. The cellular effects of angiotensin II (AngII) are mediated by distinct receptor subtypes, the AngII type 1 and type 2 receptors (AT2R) [2, 3]. Although the roles of AT2R are still obscure, this receptor is activated in various pathophysiological conditions and works against the type 1 receptor [2, 3]. While hypertension is often associated with obesity and lipid disorders, the AT2R can mediate, in part, the action of

AngII on adipocyte differentiation [4] and on the development of hypertension [5]. AngII also participates in high-density lipoprotein (HDL) homeostasis in adipose tissue [6].

The AT2R gene is located on the X chromosome (q22–23), and a few studies have reported the association between the AT2R gene adenine/cytosine (A/C)-3123 polymorphism and cardiometabolic diseases [5, 7–9]. Whereas no apparent association has been reported between this polymorphism and the hypertensive state [7], more A-allele carriers have been reported in hypertensive subjects than in controls [5]. It is of interest that A-allele carriers are significantly associated with the occurrence of myocardial infarction among nonhypertensive subjects, although this association is not seen among hypertensive subjects [5]. On the other hand, there is also the observation that the A allele could be associated with better glycemic control and obesity traits [8, 9]. This observation implies a need for more research on the relationship of this polymorphism with various cardiometabolic factors in considering the hypertensive state. The present study aimed to investigate the association between the AT2R A/C3124 polymorphism and cardiometabolic variables in subjects with and without hypertension.

Subjects and Methods

A total of 161 community-dwelling Japanese women were recruited during regular health checkups and health education classes. Eligible subjects were basically asymptomatic and not taking any medication. Excluded subjects were current smokers, heavy drinkers, and those with features of diabetes mellitus (defined as fasting plasma glucose ≥ 7.0 mmol/l [10]), cardiovascular disease (CVD), malignancy, endocrine disorder, or severe kidney and liver disease. The study was approved by the Ethics Committees of Kyoto Medical Center and Tottori University, and each subject gave informed consent. The subjects were divided into hypertensive ($n = 82$, age range 50–81 years) and nonhypertensive ($n = 79$, age range 30–83 years) groups.

All data were obtained in an overnight fasted state. The body mass index (BMI) was calculated as the weight divided by the height squared. Blood pressure, systolic (SBP) and diastolic (DBP), was measured at the right arm in a seated position using a mercuric sphygmomanometer with appropriately sized cuffs in conformity with the expert guidelines [11]. Subjects with SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg were defined as cases of hypertension [11]. Plasma glucose, serum total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were measured by enzymatic methods. The level of low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation [12]. Plasma insulin was measured by enzyme-linked immunosorbent assay (TOSOH Co. Ltd., Tokyo, Japan). The index of homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: (plasma glucose \times insulin)/22.5 [13].

DNA was extracted from the subjects' buccal mucosa cells obtained using cytobrushes, and genotypes were determined by an intercalation-mediated fluorescent allele-specific PCR method, based on a previous report [5].

Data are expressed as means \pm standard deviation or medians with interquartile range. The genotype and allele frequencies for Hardy-Weinberg equilibrium were examined using a χ^2 test. Differences between the groups were compared using unpaired *t* tests. A general linear model for HDL-C (as a dependent variable) was used to examine the influence of carrying the A allele (as a fixed variable) with adjustments for multiple variables. SBP and LDL-C were the only variables entered into the multivariate-adjusted models because of the close correlation between SBP and DBP, as well as between TC and LDL-C. The TG, insulin, and HOMA-IR values were log transformed because of their skewed distributions. A $p < 0.05$ was considered statistically significant.

Results

In the whole population, the mean/median levels of the variables were as follows: age, 66 ± 11 years; BMI, 24.2 ± 3.3 kg/m²; SBP, 139 ± 19 mm Hg; DBP, 78 ± 11 mm Hg; TC, 5.01 ± 0.84 mmol/l; LDL-C, 3.04 ± 0.72 mmol/l; HDL-C, 1.48 ± 0.35 mmol/l; TG, 0.95 mmol/l (0.77–1.27); glucose, 5.15 ± 0.64 mmol/l; insulin, 6.6 μ U/l (4.3–9.5), and HOMA-IR 1.45 (0.94–2.32). The hypertensive subjects were older than the nonhypertensive subjects (63 ± 13 years) and the difference was statistically significant ($p < 0.01$). The following parameters were higher in hypertensive subjects than in nonhypertensive subjects as follows: BMI: 24.8 ± 3.4 versus 23.7 ± 3.0 kg/m², $p = 0.03$; SBP: 153 ± 12 versus 124 ± 12 mm Hg, $p < 0.01$; DBP: 83 ± 11 versus 72 ± 8 mm Hg, $p < 0.01$, and TG: 1.01 mmol/l (0.87–1.36) versus 0.95 mmol/l (0.77–1.27), $p < 0.01$. The other variables did not show any significant differences between hypertensive and nonhypertensive subjects.

The distributed number of C/C, A/C, and A/A genotypes was 59, 86, and 16, respectively. The frequency of the A allele was 37%. This frequency was similar to values in earlier Japanese reports [5, 8, 9]. These frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 3.80$, $p = 0.15$). There was no significant difference in allele and genotype frequencies ($p = 0.80$, $p = 0.28$) between hypertensive and nonhypertensive subjects.

The analysis based on the genotypes revealed that, among the non-A-allele carriers, hypertensive subjects were significantly older ($p < 0.01$) and had significantly higher BMI ($p = 0.02$), SBP ($p < 0.01$), and DBP ($p < 0.01$), while they had significantly lower HDL-C levels ($p = 0.01$), compared to nonhypertensive subjects (table 1).

Table 1. Clinical characteristics associated with the AT2R gene A/C3124 polymorphism and hypertension

	All subjects		Hypertensive subjects		Nonhypertensive subjects	
	C/C (n = 59)	A/C + A/A (n = 102)	C/C (n = 33)	A/C + A/A (n = 49)	C/C (n = 26)	A/C + A/A (n = 53)
Age, years	67 ± 11	66 ± 11	69 ± 7	69 ± 8	62 ± 14 ^a	64 ± 12 ^b
BMI, kg/m ²	24.0 ± 3.6	24.4 ± 3.1	24.9 ± 3.5	24.6 ± 3.4	22.8 ± 3.3 ^a	24.1 ± 2.9
SBP, mm Hg	141 ± 21	138 ± 17	155 ± 15	152 ± 9	123 ± 14 ^a	124 ± 11 ^b
DBP, mmHg	78 ± 12	77 ± 11	84 ± 12	83 ± 10	72 ± 8 ^a	72 ± 9 ^b
Total cholesterol, mmol/l	5.01 ± 0.80	5.00 ± 0.87	4.97 ± 0.90	5.17 ± 0.78	5.07 ± 0.67	4.84 ± 0.93
LDL-C, mmol/l	3.00 ± 0.69	3.05 ± 0.73	3.03 ± 0.77	3.19 ± 0.68	2.97 ± 0.59	2.93 ± 0.77
HDL-C, mmol/l	1.53 ± 0.35	1.45 ± 0.34	1.44 ± 0.35	1.44 ± 0.30	1.66 ± 0.33 ^a	1.45 ± 0.38 [*]
TG, mmol/l	0.93 (0.74–1.22)	1.00 (0.80–1.27)	0.93 (0.78–1.21)	1.10 (0.91–1.39)	0.89 (0.66–1.32)	0.92 (0.72–1.15)
Plasma glucose, mmol/l	5.22 ± 0.68	5.11 ± 0.61	5.09 ± 0.47	5.11 ± 0.50	5.38 ± 0.86	5.13 ± 0.70
Insulin, μU/l	6.1 (4.2–8.9)	6.8 (4.3–9.9)	7.1 (4.3–9.4)	7.7 (4.4–9.9)	5.8 (4.0–7.7)	6.6 (4.2–9.9)
HOMA-IR	1.42 (0.97–2.31)	1.59 (0.91–2.51)	1.45 (0.94–2.26)	1.69 (0.98–2.27)	1.29 (0.96–2.45)	1.39 (0.88–2.59)

Values are expressed as means ± standard deviation for parametrically distributed variables and as medians (interquartile range) for nonparametrically distributed variables. TG, insulin, and HOMA-IR were analyzed after log transformation because of their skewed distribution. The p value was based on the comparison between the groups with and without the A allele using an unpaired t test.

^{a, b} p < 0.05 (hypertensive vs. nonhypertensive subjects within the C/C or A/C + A/A genotype groups).

^{*} p < 0.05 (C/C vs. A/C + A/A within all subjects and hypertensive or nonhypertensive subject groups).

Among the A-allele carriers, hypertensive subjects were significantly older ($p = 0.03$) and had significantly higher levels of SBP ($p < 0.01$) and DBP ($p < 0.01$) than nonhypertensive subjects.

The analysis based on the state of hypertension revealed that, among nonhypertensive subjects, A-allele carriers had significantly lower HDL-C levels than non-A-allele carriers did ($p = 0.02$), while there were no significant differences in other variables between A-allele carriers and non-A-allele carriers (table 1). Among hypertensive subjects, there were no significant differences in any variable between A-allele carriers and non-A-allele carriers. The influence of the A allele on HDL-C remained independently significant among nonhypertensive subjects ($F = 3.99$, $p = 0.04$) when basic confounding variables (age and BMI) were adjusted in a general linear model analysis. The influence of the A allele on HDL-C continued to be similarly significant ($F = 4.80$, $p = 0.03$) after adjusting for the above mentioned basic confounders plus SBP, LDL-C, TG, and HOMA-IR.

Discussion

The present study demonstrated that A-allele carriers had significantly lower HDL-C levels than the non-A-allele carriers among nonhypertensive women, while this association was not observed among hypertensive wom-

en. This result suggests that the A/C3124 polymorphism may be a marker associated with HDL metabolism according to the absence/presence of hypertension. This finding is also noteworthy because the renin-angiotensin system and HDL metabolism are both promising targets to control the development of cardiometabolic diseases. The present study showed no difference in the genetic distribution of the A/C3124 polymorphism between hypertensive and nonhypertensive subjects, and this appears to be consistent with an earlier study [7]. One study, however, showed the A allele to be associated with the occurrence of myocardial infarction in nonhypertensive subjects, but not in hypertensive subjects [5]. This indicates that there is a difference in the association of the A/C3124 polymorphism with cardiometabolic factors associated with pathophysiological changes resulting from the hypertensive state. As a result, the findings of the present study may extend prior knowledge [5, 7] and provide new hints for research on the relationship of the AT2R gene polymorphism and cardiometabolic conditions.

The detailed mechanism(s) of these results remain to be determined. AngII enhances HDL clearance (decreasing circulating HDL-C levels) in adipose tissue and/or the liver (i.e. via the scavenger receptor type-BI [6]). The A/C3124 polymorphism may be associated with this pathway, and some pathophysiologies of hypertension can modulate this association. The association between

HDL-C and the A/C3124 polymorphism, observed in the present study, was unaffected by adjustment for the BMI and HOMA-IR. The BMI and insulin resistance levels may not necessarily reflect adipocyte functions and, if so, the above mentioned modulation may occur at cellular levels. In addition, a nonsignificant influence of the A/C3124 polymorphism on the LDL-C level was observed in this study. Therefore, this polymorphism does not seem to modulate the pathway of cholesterol transfer between HDL and LDL particles. Furthermore, the A/C3124 polymorphism is located in untranslated regions of the X chromosome, so linkage disequilibrium in a functional variant of the same or a different gene must be considered in the association study [7]. Future research is warranted for biological explanations of the results.

Association studies of large populations and genome-wide association studies have identified various genetic variations for circulating HDL-C levels (i.e. these variations are found not only in apolipoproteins and enzyme proteins that have been previously known but also in proteins that have not been identified by hypothesis-driven approaches) [14]. These findings have also paved the way for the identification of a novel metabolic pathway for HDL-C and its relationship to CVD, though the results of many studies have been often inconsistent [14]. The gene polymorphisms related to the renin-angiotensin system have not been included in the candidate genetic variations for HDL-C [14], but a difference in HDL-C levels between individuals with different genotypes of angiotensin-converting enzyme was also seen in a population-based study [15].

This study had the limitation of a small sample size, a population restricted to women, and a cross-sectional design. In particular, the results from this small number of samples need to be replicated for confirmation of the findings. No data on CVD-related outcomes were available. The circulating HDL-C levels can also be modified by lifestyle-related factors [14], and the present study excluded subjects who were current smokers, heavy drinkers, and used any medications, but subjects' exercise habits were not assessed. More studies with larger sample sizes, various populations, and longer observations, as well as including various measures, will be conducted in the future.

Conclusion

Collectively, A-allele carriers had significantly lower HDL-C levels than non-A-allele carriers among nonhypertensive women. This suggests that the A/C3124 polymorphism may be a polymorphic marker of HDL metabolism by hypertension. This was a small study, so further research is called for to confirm these findings.

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