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The relationship between adiponectin, an adiponectin gene polymorphism, and high-density lipoprotein particle size: from the Mima study

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ABSTRACT

This study examined the association among serum adiponectin levels, a single nucleotide polymorphism (SNP) of the adiponectin gene, and the size of serum high-density lipoprotein (HDL) particles in a general population. A total of 275 subjects were examined as part of the community-based Mima study. Serum adiponectin levels were measured with an enzyme-linked immunosorbent assay. Serum small-sized HDL was measured with the electrophoretic separation of lipoproteins using the Lipoprint system. Single nucleotide polymorphism G276T (rs1501299, SNP276) of the adiponectin gene was determined with a fluorescent allele-specific DNA primer assay system. Age- and sex-adjusted correlation test revealed a significant inverse relationship between small-sized HDL and adiponectin levels ($r = -0.236$, $P < .001$). More percentages of small-sized HDL were observed in the subjects with the SNP276 G/G and G/T genotypes than in those with the T/T genotype (5.5% ± 5.0% vs 3.0% ± 2.9%, $P = .016$). In a multiple regression analysis, small-sized HDL was significantly and independently correlated with triglycerides levels ($\beta = 0.133$, $P = .030$), adiponectin levels ($\beta = -0.242$, $P < .001$), and the SNP276 G allele ($\beta = -0.142$, $P = .014$). Our findings indicated that adiponectin and SNP276 of the adiponectin gene may modify the size of HDL particles.

1. Introduction

Low levels of high-density lipoprotein cholesterol (HDL-C) in the circulation increase the risk of coronary artery disease (CAD) [1]. Japanese have higher levels of HDL-C than Westerners [2,3], and some studies on Japanese populations have demonstrated an inverse relationship between HDL-C levels and CAD risk [4,5]. However, not only the quantity but also the quality of HDL is important in regard to atherogenesis. Small-sized HDL has been shown to be a hallmark of the metabolic

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dyslipidemia observed among patients with intraabdominal obesity [6]. Improvements in lifestyle, including alcohol intake, smoking, and exercise habits, have been associated with not only HDL-C levels but also HDL size [7]. Large-sized HDL has been positively associated with adiponectin levels [8]. In the field of epidemiology, it has been suggested that large-sized HDL has potent antiatherogenic properties [9]. Therefore, HDL particle size may be crucial as a quantitative marker [10].

Adiponectin plays an important role in various lifestyle-related diseases, such as obesity, hypertension, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome, leading to the development of CAD [11,12]. Adiponectin levels are positively correlated with HDL-C levels [13]. Studies have demonstrated that adiponectin accelerated reverse cholesterol transport by increasing the amount of HDL in the liver [14], suggesting that adiponectin enhances the adenosine triphosphate–binding cassette transporter A1 (ABCA1) pathway and apolipoprotein (apo) A-1 synthesis [14].

The adiponectin gene has several single nucleotide polymorphisms (SNPs) [15]. Subjects with the major G allele at position 276 in the adiponectin gene (rs1501299) had an approximately 2-fold risk for developing type 2 diabetes mellitus compared with minor T allele carriers [16]. In some studies on the SNP276 of the adiponectin gene, there were no differences shown in the HDL-C levels between subjects with the SNP276 G/G or G/T genotype and those with the T/T genotype [17,18]. Moreover, no studies have been conducted on the SNPs in the adiponectin gene and the quality of HDL. In this study, we investigated the relationship between adiponectin, the adiponectin gene SNP276, and HDL particle size.

2. Subjects and methods

2.1. Subjects

A total of 275 Japanese community-dwelling subjects in Mima city, Tokushima prefecture (116 men, 159 women; aged 27-88 years) were enrolled in this study. We included subjects who were apparently healthy without any known medical history of chronic diseases. The study protocol was approved by the Ethics Committee of the National Hospital Organization, Kyoto Medical Center. All of the subjects signed an informed consent form after being fully informed about all aspects of the study before they were enrolled in the study. After an overnight fast, body weight and height were measured using a body fat analyzer (OMRON, Osaka, Japan). Blood pressure was measured 3 times, at 10-minute intervals, using a mercury sphygmomanometer by nurses or hygienists. Venous blood samples were then drawn for blood tests. Blood glucose concentrations were measured by the hexokinase method (SHINO-TEST, Tokyo, Japan), and serum insulin concentrations were assayed by chemiluminescent immunoassay (Bayer Medical, Tokyo, Japan). Serum total cholesterol (TC) (Wako Pure Chemical Industries, Tokyo, Japan), HDL-C, and triglycerides (TG) (DAIICHI PURE CHEMICALS, Tokyo, Japan) levels were determined by enzymatic methods. The medical history and lifestyle items were confirmed by a public heath questionnaire or interview.

2.2. Measurement of small-sized HDL

Serum small-sized HDL was detected in the HDL subfractions using the Lipoprint system (Quantimetrix, Redondo Beach, CA) [19]. All of the HDL subfractions were calculated based on a flotation rate. Subfractions HDL-8, HDL-9, and HDL-10 were defined as small-sized HDL.

2.3. Genetic analysis

The SNP276 of adiponectin was amplified using the polymerase chain reaction with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-TAG GCC TTA GTT AAT AAT GAA TxC C-3′) or Texas red (5′-CTA GGC CTT AGT TAA TAA TGA ATx AC-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-CAT CAC AGA CCT CCT ACA CTG ATA-3′) (Toyobo Gene Analysis, Tsuruga, Japan). The reaction profile was an initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 68°C for 30 seconds; and a final extension at 68°C for 2 minutes. The amplified DNA was measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan). We had all genotypes checked by 2 independent investigators, and only those showing concordance were used.

2.4. Statistical analysis

Data were expressed as means ± SD. The present study was designed to detect a difference in means equivalent to 5% of small-sized HDL with a standard deviation of 5%. Thus, the sample size required was 198 with an error of 5%, with 80% power ($\beta = 20\%$) at the 2-tailed 5% significance level [20]. The χ^2 test was performed to test for any deviation from the Hardy-Weinberg equilibrium. The differences between each group (G/G, G/T, and T/T) were estimated by the analysis of variance and the χ^2 test. The differences between the groups, the G/G + G/T genotypes and the T/T genotype (dominant model) or the G/G genotype and G/T + T/T genotypes (recessive model) in SNP276 of the adiponectin gene, were estimated by using Student unpaired t test, the Mann-Whitney U test, and the χ^2 test. A correlation test adjusted for age and sex was used between parameters. A multiple regression analysis controlled for age, sex, body mass index (BMI), TC, TG, homeostasis model assessment of insulin resistance (HOMA-IR), adiponectin, smoking, alcohol intake, and the SNP276 G allele was performed to explore the correlation with small-sized HDL. Insulin, adiponectin, and TG levels were log-transformed. $P < .05$ was accepted as statistically significant. Statistical analyses were performed with the Statistical Package of Social Science (SPSS for Windows, version 11.0; SPSS, Chicago, IL).

3. Results

The distribution of the G/G, G/T, and T/T genotypes was 135, 116, and 24, respectively. The frequency of the G allele was 0.702. This distribution was in accordance with the Hardy-Weinberg equilibrium ($P = .996$).

The clinical and biochemical characteristics of all of the subjects are listed in Table 1. There were no significant

Table 1 – Clinical and biological characteristics of subjects according to SNP276 of the adiponectin gene

Parameters	Genotype			P value[a]	P value[b]	
					Dominant model	Recessive model
	G/G	G/T	T/T		G/G + G/T vs T/T	G/G vs G/T + T/T
n	135	116	24			
Age (y)	65 ± 15	67 ± 10	66 ± 11	.207	.905	.080
Sex (men/women)	62/73	47/69	8/16	.436	.393	.275
Alcohol intake (every day, %)	17.8	14.7	12.5	.690	.776	.325
Smoking (current, %)	25.2	22.4	12.5	.337	.308	.471
Exercise habit (<2×/wk, %)	74.1	73.3	69.6	.717	.625	.056
BMI (kg/m^2)	24.0 ± 3.1	24.0 ± 3.0	24.6 ± 3.0	.663	.345	.844
Systolic blood pressure (mm Hg)	139 ± 20	139 ± 19	136 ± 23	.949	.524	.907
Diastolic blood pressure (mm Hg)	78 ± 11	76 ± 12	76 ± 13	.427	.360	.194
Fasting plasma glucose (mmol/L)	5.6 ± 1.8	5.6 ± 1.5	5.8 ± 2.2	.720	.827	.751
Fasting insulin (pmol/L)	43 ± 36	52 ± 64	42 ± 24	.452	.992	.338
HOMA-IR	1.9 ± 2.6	2.3 ± 3.7	1.8 ± 1.2	.623	.799	.480
TG (mmol/L)	1.15 ± 0.65	1.10 ± 0.52	1.14 ± 0.39	.660	.334	.389
TC (mmol/L)	4.84 ± 0.93	4.86 ± 0.93	4.63 ± 0.83	.626	.258	.908
HDL-C (mmol/L)	1.37 ± 0.34	1.45 ± 0.41	1.32 ± 0.36	.230	.276	.213
Small-sized HDL (%)	5.6 ± 4.7	5.5 ± 5.4	3.0 ± 2.9	.057	.013	.187
Adiponectin (μg/mL)	8.6 ± 6.1	8.5 ± 5.4	8.1 ± 6.1	.992	.611	.910

Data are means ± SD.
[a] *P* values were obtained using analysis of variance for continuous variables or using the χ^2 test for categorical variables.
[b] *P* values were obtained using Student unpaired *t* test, Mann-Whitney *U* test, and χ^2 test in 2 models: the dominant model (G/G + G/T vs T/T) or recessive model (G/G vs G/T + T/T).

differences among the genotypes. Assuming an additive genetic model, carriers of the minor T allele of SNP276 had decreased, but not significantly, on the measurement of small-sized HDL (per allele effect [β] = –0.79% [–1.69%, +0.10%], *P* = .083). In the dominant model (G/G + G/T vs T/T), there were no differences in sex, age, alcohol intake, smoking, exercise habits, BMI, systolic blood pressure, diastolic blood pressure, fasting plasma glucose concentration, fasting insulin levels, HOMA-IR, TC, TG, HDL-C, and adiponectin levels. More small-sized HDL was observed in the subjects with the SNP276 G/G or G/T genotype than in those with the T/T genotype (5.5% ± 5.0% vs 3.0% ± 2.9%, *P* = .013, Table 1), although there was no difference in the adiponectin levels between the groups.

The sex- and age-adjusted correlation test revealed that there was a significant positive correlation between small-sized HDL and TC or (log-)TG (*r* = 0.195, *P* = .008; *r* = 0.161, *P* = .001, respectively) (Table 2). There was also a significant inverse correlation between small-sized HDL and fasting glucose or (log-)adiponectin levels (*r* = –0.146, *P* = .016; *r* = –0.236, *P* < .001, respectively) (Table 2).

Moreover, a multiple regression analysis revealed that small-sized HDL was significantly and independently correlated with TG, adiponectin, and SNP276 G allele (Table 3).

Table 2 – Pearson correlation test between small-sized HDL and other parameters

Parameter	*r*	*P* value
BMI	0.118	.053
Systolic blood pressure	0.019	.759
Diastolic blood pressure	0.074	.218
Fasting plasma glucose	–0.146	.016
Fasting insulin	–0.023	.711
HOMA-IR	–0.048	.427
TG	0.161	.001
TC	0.195	.008
HDL-C	–0.075	.219
Adiponectin	–0.236	<.001

4. Discussion

This study disclosed that small-sized HDL was significantly and independently correlated with adiponectin levels and the G allele of SNP276. This is the first indication that adiponectin and one of its SNPs may be associated with HDL particle size.

Weiss et al [8] reported that large-sized HDL is positively associated with adiponectin levels. The results of the present

Table 3 – A multiple regression analysis for small-sized HDL

Dependent variable	Independent variables	*B*	SE	95% CI	*P* value
Small-sized HDL					
Model 1	Adiponectin	–0.24	0.050	–0.34 to –0.14	<.001
Model 2	Adiponectin	–0.24	0.049	–0.34 to –0.14	<.001
	Adiponectin gene SNP276	–2.44	0.984	–4.38 to –0.50	.014
Model 3	Adiponectin	–0.20	0.052	–0.30 to –0.10	<.001
	Adiponectin gene SNP276	–2.44	0.978	–4.37 to –0.52	.013
	TG	0.01	0.006	0.00 to 0.02	.032

The following parameters were used: age, sex, BMI, TC, TG, HOMA-IR, adiponectin, smoking, alcohol intake, and adiponectin gene SNP276 (G/G and G/T = 0, T/T = 1). SE indicates standard error; CI, confidence interval.

study also showed a significant positive correlation between adiponectin and HDL-C levels ($r = 0.124$, $P = .041$) and large-sized HDL ($r = 0.290$, $P < .001$). Although the association of adiponectin with HDL particles could not be explained, it might be understood in regards to the role of HDL in removing cholesterol from cells and transporting it to the liver. Adiponectin increased both the messenger RNA and protein levels of ABCA1 and enhanced the messenger RNA level of apo A-1 in HepG2 cells [14]. In addition, ABCA1 expression in the liver and apo A-1 expression in plasma and the liver were reduced in adiponectin knockout mice [21]. Adiponectin may thus alter the size of HDL particles through the ABCA1 or apo A-1 pathway. Chan et al [22] reported that adiponectin may be a key factor in very low-density lipoprotein (VLDL) metabolism in nonobese men. Therefore, there is another reverse pathway of transport involving the cholesteryl ester transfer protein, where cholesterol esters are transferred from HDL particles to TG-rich lipoproteins such as VLDL, and adiponectin can increase lipoprotein lipase activity and decrease VLDL-TG levels. Therefore, altered lipoprotein lipase activity due to a change in adiponectin levels may also be relevant to the metabolism of HDL particles.

SNP276 in the adiponectin gene has been frequently described as associated with obesity and CADs. This SNP has also been concomitantly associated with greater insulin resistance. Serum adiponectin levels were significantly lower in subjects with the G/G or G/T genotype than those with the T/T genotype [23], but not all [17]. Chiodini et al [24] reported that subjects with the SNP276 T/T genotype may be protected from the risk of myocardial infarction in an Italian population. In addition to HDL-C measurements, the HDL particle size is superior to HDL-C in cardiovascular risk assessment [10]. Moreover, based on our results, the association of SNP276 with small-sized HDL may be explained by linkage disequilibrium in a functional variant of the same or a different gene. More studies are necessary to clarify whether this SNP affects the size of HDL particles.

There were several limitations to our study. First, a cross-sectional design has inherent problems in defining causality. Second, with the measurement system used, each subfraction is defined by a percentage area only, although absolute values are of interest. Third, we did not check the 45-276 haplotype. Further research with comparisons to other methodologies to assess the quality of HDL may strengthen our data.

In summary, we examined the relationships between adiponectin, SNP276 of the adiponectin gene, and the HDL particle size in a general population. Small-sized HDL was found to be significantly and independently correlated with adiponectin levels and the G allele of SNP276, suggesting that both adiponectin and SNP276 may influence the size of HDL particles. Understanding the mechanisms involved may provide important information for both the prevention and management of CAD.

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Conflict of Interest

None to disclose.

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