Original Article

CDH13 Polymorphisms are Associated with Adiponectin Levels and Metabolic Syndrome Traits Independently of Visceral Fat Mass

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Aim: Visceral fat accumulation contributes to the development of metabolic syndrome. As visceral fat accumulation increases, adiponectin levels decrease; therefore, adiponectin provides a link between visceral fat accumulation and metabolic disorders. Genome-wide association studies (GWASs) have identified genetic variations in the cadherin 13 (*CDH13*) gene that are associated with adiponectin levels.

Methods: We investigated whether single nucleotide polymorphisms (SNPs) in *CDH13* was associated with adiponectin levels and metabolic syndrome traits independent of the visceral fat area (VFA), as measured using computed tomography (CT) in 945 Japanese individuals.

Results: We found that three *CDH13* SNPs reported by recent GWASs (i.e., rs3865188, rs4783244, and rs12051272) were significantly associated with higher adiponectin levels ($P < 1 \times 10^{-14}$), even after adjustment for VFA. However, these adiponectin-inducing alleles of *CDH13* SNPs were significantly associated with traits consistent with deteriorating metabolic symptoms, such as higher fasting insulin, homeostasis model assessment–insulin resistance (HOMA-IR) scores, and triglycerides and lower high-density lipoprotein (HDL)-cholesterol levels, similar to increasing VFA and decreasing adiponectin levels.

Conclusion: These results suggested that *CDH13* SNPs cause an adiponectin-resistant status to compensate for increasing adiponectin levels and could result in the deterioration of metabolic syndrome traits.

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Key words: *CDH13*, Single nucleotide polymorphism, Adiponectin, Visceral fat area, Metabolic syndrome

Introduction

Excess accumulation of adipose tissue, particularly visceral fat, contributes to the development of insulin resistance, resulting in symptoms characteristic of metabolic syndrome, including type 2 diabetes, dyslipidemia, and hypertension^{1, 2}. In parallel with an increase in visceral fat, circulating adiponectin levels decrease, causing insulin resistance, type 2 diabetes, and cardiovascular disease, thereby providing a critical link between visceral fat accumulation and metabolic syndrome²⁻⁸. However, in addition to visceral fat accumulation, genetic variations may affect adiponectin levels, as demonstrated in recent genome-wide association studies (GWASs)⁹⁻¹⁷. One of the most important genetic variations determining adiponectin levels is located in the cadherin 13 (*CDH13*) gene, which encodes an adiponectin receptor¹⁸.

Visceral fat is evaluated by measuring waist circumference, waist-hip ratio, bioelectrical impedance, or more precisely, the visceral fat area (VFA), determined by computed tomography (CT)². Adiponectin levels are more strongly correlated with VFA than subcutaneous fat area (SFA)^{8, 19}. Although some studies have reported an association between polymorphisms in the *CDH13* and metabolic syndrome traits^{20, 21}, these studies did not measure VFA. Therefore, despite the potential link among VFA, adiponectin levels, and *CDH13* polymorphisms, the effects of *CDH13* genetic polymorphisms on circulating adiponectin levels and metabolic syndrome traits in individuals with varying VFAs remain unclear.

Aim

We investigated whether the effects of *CDH13* polymorphisms on adiponectin levels and metabolic syndrome traits are dependent on VFA.

Methods

Participants

Japanese participants ($n=945$) were recruited for this study. Patients with a disease (such as cancer, renal disease, cardiovascular disease, and hepatic failure) or receiving medication (i.e., insulin, thiazolidinedione, corticosteroids, or chemotherapy) that would be

expected to affect body weight or adiponectin levels were excluded. The participants agreed to undergo CT to determine VFA and SFA at the umbilical level (L4-L5), as previously reported²². VFA and SFA were calculated using the FatScan software program (N2system, Osaka, Japan)²². Clinical data, which were recorded at the first visit to the hospital, are summarized in **Table 1**. Homeostasis model assessment-insulin resistance (HOMA-IR) scores were assessed as fasting insulin ($\mu\text{U/mL}$) $\times$ fasting plasma glucose (mg/dL)/405. The recruitment and study protocols were conducted in compliance with the Declaration of Helsinki, and all participants provided written informed consent. The study was approved by Kyoto University (G374).

DNA Extraction and Single Nucleotide Polymorphism (SNP) Genotyping

Genomic DNA was extracted from blood samples that were collected from each participant using a Genomix kit (Talent Srl, Trieste, Italy). We selected three SNPs (rs3865188, rs4783244, and rs12051272) in the *CDH13* gene that were recently identified by GWASs^{9, 12-15} and constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for these SNPs. The SNPs were genotyped using Invader assays, as previously described²³, and used for analysis. The success rate of these assays was $>99.7\%$.

Measurement of Plasma Adiponectin Levels

Plasma total adiponectin levels were measured using an enzyme-linked immunosorbent assay (ELISA; human adiponectin ELISA kit; Otsuka Pharmaceutical Co., Tokushima, Japan).

Statistical Analysis

For the additive model, we characterized the genotypes as 0, 1, or 2, depending on the number of copies of the tested alleles. Tested alleles were defined as previously identified adiponectin-inducing alleles^{9, 12-15}. Multiple linear regression analyses were performed to test the independent effects of each allele from each SNP on adiponectin and metabolic syndrome traits, accounting for the effects of other variables [i.e., age, sex, body mass index (BMI), VFA, and SFA]. Values for BMI, VFA, SFA, fasting glucose, fasting insulin, HOMA-IR, triglycerides, and adiponectin were logarithmically transformed before performing multiple linear regression analysis. Linkage disequilibrium was calculated using Haploview²⁴.

A structural equation modeling (SEM) analysis was used to assess the contribution of *CDH13* SNPs to the circulating adiponectin levels and metabolic

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Table 1. Clinical characteristics of the participants

	Men	Women	Total
<i>n</i>	412	533	945
Age (year)	50.6 ± 11.1	51.8 ± 10.4	51.3 ± 10.7
BMI (kg/m ²)	29.5 ± 5.0	27.3 ± 4.4	28.2 ± 4.8
VFA (cm ²)	161.5 ± 66.9	93.6 ± 50.1	123.2 ± 67.0
SFA (cm ²)	202.3 ± 98.5	239.0 ± 89.0	223.0 ± 94.9
V/S (cm ²)	0.9 ± 0.4	0.4 ± 0.2	0.6 ± 0.4
Fasting plasma glucose (mg/dL)	103.9 ± 29.0	97.5 ± 20.7	100.3 ± 24.9
Insulin (μU/mL)	11.7 ± 14.8	8.9 ± 8.1	10.1 ± 11.5
HOMA-IR	3.2 ± 4.7	2.2 ± 2.2	2.6 ± 3.6
Total cholesterol (mg/dL)	213.3 ± 36.8	221.8 ± 36.6	218.1 ± 36.9
Triglycerides (mg/dL)	164.1 ± 158.4	108.1 ± 76.2	132.5 ± 122.3
HDL-cholesterol (mg/dL)	53.2 ± 14.0	62.7 ± 15.5	58.6 ± 15.6
Systolic blood pressure (mmHg)	132.4 ± 17.0	130.5 ± 19.0	131.3 ± 18.2
Diastolic blood pressure (mmHg)	86.3 ± 12.3	81.1 ± 10.8	83.4 ± 11.7
Adiponectin (μg/mL)	6.1 ± 3.1	9.8 ± 5.0	8.2 ± 4.7

HOMA-IR was assessed as fasting insulin (μU/mL) × fasting plasma glucose (mg/dL)/405.

syndrome, as described in a previous report²⁵), with some modifications. The path diagram is shown in **Fig. 1**. The *CDH13* rs12051272 genotype, visceral fat accumulation, and decreased adiponectin levels were hypothesized to be upstream of the clustering of metabolic abnormalities (risk clustering). Risk clustering was assumed to be measured by fasting glucose, HOMA-IR, triglycerides, high-density lipoprotein (HDL)-cholesterol, and systolic and diastolic blood pressures. The effects of age and sex on VFA and adiponectin levels were added in this path diagram.

All statistical analyses were performed using R software (<http://www.r-project.org/>). SEM analysis was performed using the lavaan package in R²⁶. Differences with *P* values of <0.016 (0.05/3 SNPs) were considered statistically significant.

Results

Effects of SNPs, BMI, VFA, and SFA on Adiponectin Levels

The clinical characteristics and genotypes of the participants are shown in **Tables 1** and **2**. All SNPs were in Hardy-Weinberg equilibrium (*P* > 0.5). The frequencies of adiponectin-inducing alleles did not diverge from those reported in the HapMap database. As previously reported, adiponectin-inducing alleles of the three SNPs (rs3865188, rs4783244, and rs12051272) were associated with higher adiponectin levels (**Table 2**). Multiple linear regression analyses indicated that these associations were significant after

adjustment for age, sex, and anthropometric parameters (BMI, VFA, and SFA; **Table 3**). Among the anthropometric parameters, VFA was most strongly associated with adiponectin levels, even after adjustment for BMI. Each adiponectin-inducing allele of the three SNPs showed stronger associations with adiponectin levels than BMI and SFA (*P* < 1 × 10⁻¹⁴). Therefore, we used VFA, instead of BMI, as an explanatory variable in the following analyses of allelic effects on metabolic syndrome traits.

Effects of SNPs, VFA, and Adiponectin Levels on Metabolic Syndrome Traits

Further, we examined the allelic effect of SNPs on metabolic syndrome traits (**Table 4**). Higher VFA and lower adiponectin levels were significantly associated with higher fasting plasma glucose, insulin, HOMA-IR, and triglycerides and lower HDL-cholesterol levels, confirming the results of several previous reports^{3, 6-8}). Although adiponectin-inducing alleles of the three SNPs were associated with higher adiponectin levels, these alleles were significantly associated with higher fasting insulin, HOMA-IR, and triglycerides and lower HDL-cholesterol levels. The effect of these SNPs (i.e., promoting deterioration of metabolic syndrome traits) was unrelated to higher adiponectin and lower VFA levels. Thus, the allelic effects of the three SNPs on adiponectin and metabolic syndrome traits were contradictory. The adiponectin-inducing alleles caused deterioration of metabolic syndrome traits, confirming the results of a previous report²¹).

Fig. 1. Effects of the *CDH13* rs12051272 genotype, adiponectin levels, and VFA on metabolic syndrome

Risk clustering was a latent variable measured by plasma glucose (PG), HOMA-IR, triglycerides (TG), HDL-cholesterol (HDL), and systolic (SBP) and diastolic (DBP) blood pressures. Rectangles are observed variables, and e₁ to e₉ are error variables. Each value on the arrow indicates the standardized regression weight.

Table 2. Genotypic characteristics of participants

SNP	rs3865188			rs4783244			rs12051272		
	AA*	AT	TT	CC*	CA	AA	GG*	GT	TT
<i>n</i>	449	397	97	451	400	92	452	402	91
Age (year)	51.1 ± 10.9	51.4 ± 10.8	51.4 ± 9.5	51.0 ± 10.9	51.5 ± 10.6	51.5 ± 9.9	51.0 ± 10.9	51.5 ± 10.7	51.7 ± 9.7
BMI (kg/m ²)	28.3 ± 4.9	28.1 ± 4.8	28.3 ± 4.2	28.4 ± 5.0	28.1 ± 4.7	28.2 ± 4.3	28.4 ± 5.0	28.1 ± 4.7	28.3 ± 4.2
VFA (cm ²)	122.6 ± 69.1	124.8 ± 67.4	120.2 ± 55.9	123.7 ± 69.4	123.2 ± 66.9	120.9 ± 56.1	123.7 ± 69.7	123.1 ± 66.4	121.2 ± 56.4
SFA (cm ²)	228.1 ± 98.1	215.8 ± 89.1	228.2 ± 102.6	228.5 ± 98.2	216.7 ± 89	222.8 ± 103.8	228.7 ± 98.4	216.1 ± 89.0	225.8 ± 102.2
Plasma glucose (mg/dL)	100 ± 22.5	100.6 ± 24.3	100.3 ± 35.6	100 ± 22.4	100.5 ± 24.3	100.7 ± 36.5	100 ± 22.4	100.5 ± 24.2	101.2 ± 36.6
Insulin (μU/mL)	10.9 ± 12.7	9.7 ± 11.1	8.7 ± 6.5	10.9 ± 12.8	9.5 ± 11	8.9 ± 6.6	10.9 ± 12.8	9.5 ± 10.9	8.9 ± 6.6
HOMA-IR	2.8 ± 3.8	2.5 ± 3.5	2.2 ± 2.1	2.8 ± 3.9	2.5 ± 3.5	2.3 ± 2.2	2.8 ± 3.8	2.5 ± 3.5	2.3 ± 2.2
Total cholesterol (mg/dL)	217.5 ± 36.1	219.6 ± 37.9	214.7 ± 36.9	217.4 ± 36.2	219.9 ± 37.7	214 ± 37.4	217.6 ± 36.1	219.4 ± 37.8	214.6 ± 37.3
Triglycerides (mg/dL)	131.7 ± 92.4	136.1 ± 157.8	122.5 ± 66	130.7 ± 92	136.6 ± 157.5	123.6 ± 67.1	130.7 ± 92.0	136.6 ± 157	123.5 ± 67.1
HDL-cholesterol (mg/dL)	58.0 ± 15.3	58.9 ± 15.7	59.3 ± 16.1	58.2 ± 15.3	58.8 ± 15.6	59.2 ± 16.6	58.2 ± 15.4	58.9 ± 15.6	58.9 ± 16.5
Systolic blood pressure (mmHg)	130.9 ± 18.1	131.4 ± 17.6	133 ± 20.9	131 ± 18.3	131.2 ± 17.4	133.2 ± 20.8	131.0 ± 18.2	131.3 ± 17.5	133.3 ± 20.8
Diastolic blood pressure (mmHg)	83.5 ± 11.8	83.2 ± 11.6	83.3 ± 11.6	83.6 ± 11.9	83.2 ± 11.6	83.1 ± 11.3	83.6 ± 11.9	83.2 ± 11.6	83.1 ± 11.3
Adiponectin (μg/mL)	9.1 ± 5.1	7.5 ± 4.1	6.4 ± 3.5	9.1 ± 5.1	7.6 ± 4.2	6.3 ± 3.4	9.1 ± 5.1	7.5 ± 4.1	6.2 ± 3.5

* adiponectin-inducing allele.

Table 3. Effects of SNPs, BMI, VFA, and SFA on plasma adiponectin levels

	rs3865188		rs4783244		rs12051272	
Model 1	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>
SNP	0.075 (0.009)	3.6×10^{-15}	0.078 (0.009)	2.0×10^{-16}	0.082 (0.009)	1.5×10^{-17}
Age	0.004 (0.001)	1.5×10^{-13}	0.004 (0.001)	8.8×10^{-14}	0.004 (0.001)	5.7×10^{-14}
Sex	-0.183 (0.013)	4.8×10^{-42}	-0.184 (0.013)	1.9×10^{-42}	-0.183 (0.013)	1.5×10^{-42}
Log ₁₀ (BMI)	-0.317 (0.092)	6.2×10^{-4}	-0.323 (0.092)	4.7×10^{-4}	-0.321 (0.092)	4.8×10^{-4}
Model 2	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>
SNP	0.074 (0.009)	7.8×10^{-16}	0.079 (0.009)	1.3×10^{-17}	0.081 (0.009)	9.5×10^{-19}
Age	0.006 (0.001)	1.0×10^{-21}	0.006 (0.001)	3.1×10^{-22}	0.006 (0.001)	1.8×10^{-22}
Sex	-0.131 (0.014)	1.1×10^{-20}	-0.131 (0.014)	1.0×10^{-20}	-0.131 (0.014)	8.4×10^{-21}
Log ₁₀ (VFA)	-0.233 (0.025)	8.1×10^{-20}	-0.236 (0.025)	2.1×10^{-20}	-0.236 (0.025)	1.8×10^{-20}
Model 3	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>
SNP	0.073 (0.009)	1.0×10^{-15}	0.078 (0.009)	1.7×10^{-17}	0.081 (0.009)	1.1×10^{-18}
Age	0.006 (0.001)	4.4×10^{-23}	0.006 (0.001)	1.4×10^{-23}	0.006 (0.001)	7.4×10^{-24}
Sex	-0.128 (0.014)	1.4×10^{-19}	-0.127 (0.014)	1.2×10^{-19}	-0.127 (0.014)	1.1×10^{-19}
Log ₁₀ (VFA)	-0.282 (0.031)	1.1×10^{-18}	-0.284 (0.031)	3.7×10^{-19}	-0.284 (0.031)	3.0×10^{-19}
Log ₁₀ (BMI)	0.286 (0.111)	0.010	0.284 (0.11)	0.010	0.286 (0.11)	0.0094
Model 4	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>
SNP	0.075 (0.009)	3.7×10^{-15}	0.079 (0.009)	2.0×10^{-16}	0.082 (0.009)	1.5×10^{-17}
Age	0.004 (0.001)	5.6×10^{-14}	0.005 (0.001)	3.5×10^{-14}	0.005 (0.001)	2.1×10^{-14}
Sex	-0.202 (0.013)	2.7×10^{-49}	-0.203 (0.013)	5.0×10^{-50}	-0.202 (0.013)	5.1×10^{-50}
Log ₁₀ (SFA)	-0.090 (0.034)	0.0087	-0.094 (0.034)	0.0060	-0.092 (0.034)	0.0068
Model 5	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>
SNP	0.075 (0.009)	3.8×10^{-15}	0.078 (0.009)	2.2×10^{-16}	0.081 (0.009)	1.6×10^{-17}
Age	0.004 (0.001)	1.6×10^{-13}	0.004 (0.001)	9.8×10^{-14}	0.004 (0.001)	6.1×10^{-14}
Sex	-0.182 (0.016)	1.1×10^{-29}	-0.184 (0.016)	3.3×10^{-30}	-0.183 (0.015)	3.8×10^{-30}
Log ₁₀ (SFA)	0.005 (0.055)	0.93	-0.001 (0.055)	0.98	0.002 (0.055)	0.97
Log ₁₀ (BMI)	-0.326 (0.148)	0.028	-0.32 (0.148)	0.031	-0.326 (0.147)	0.027

The effect sizes and *P*-values were derived from linear regression analysis. Adiponectin was log-transformed for the analyses.

SEM Analysis of the Effects of SNPs on Adiponectin Levels and Metabolic Syndrome

In order to assess the effects of *CDH13* SNPs on adiponectin levels and metabolic syndrome, we performed SEM analysis. **Fig. 1** shows the results of the SNP rs12051272, which had the best genotyping success rate. *P*-values of all estimates in the path diagram were less than 0.005. The path diagram comparative fit index (CFI) was 0.634, and the root mean square error of approximation was 0.18. The rs12051272 genotype significantly increased adiponectin levels to be protective against metabolic syndrome (**Fig. 1**). On the other hand, the rs12051272 genotype significantly increased the risk clustering, prompting metabolic syndrome. SEM analysis using other SNPs (rs3865188

and rs4783244) showed similar results (data not shown).

Discussion

Adiponectin is specifically secreted from adipose tissue, and its plasma levels are decreased during visceral fat accumulation^{2-8, 19}.

In this study, we found that adiponectin levels were determined by *CDH13* polymorphisms, independent of visceral fat accumulation and subcutaneous and total fat mass. This is the first report demonstrating that the allelic effects of *CDH13* polymorphisms on adiponectin levels were as strong as those of visceral fat accumulation. Thus, our data suggested that the clinical study of the metabolic syndrome

Table 4. Effects of SNP, age, sex, VFA, and adiponectin levels on metabolic syndrome traits

SNP ID	rs3865188		rs4783244		rs12051272	
	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>	β (s.e.)	<i>P</i>
Plasma glucose						
SNP	0.005 (0.004)	0.23	0.005 (0.004)	0.23	0.004 (0.004)	0.29
Age	0.001 (0.000)	4.7×10^{-5}	0.001 (0.000)	4.2×10^{-5}	0.001 (0.000)	4.6×10^{-5}
Sex	-0.002 (0.006)	0.73	-0.002 (0.006)	0.74	-0.002 (0.006)	0.70
Log ₁₀ (VFA)	0.068 (0.011)	1.4×10^{-9}	0.067 (0.011)	2.0×10^{-9}	0.067 (0.011)	1.6×10^{-9}
Log ₁₀ (adiponectin)	-0.047 (0.014)	6.8×10^{-4}	-0.047 (0.014)	6.6×10^{-4}	-0.047 (0.014)	6.8×10^{-4}
Insulin						
SNP	0.048 (0.015)	0.0012	0.045 (0.015)	0.0026	0.046 (0.015)	0.0020
Age	-0.004 (0.001)	7.7×10^{-6}	-0.004 (0.001)	8.9×10^{-6}	-0.004 (0.001)	1.0×10^{-5}
Sex	-0.071 (0.023)	0.0019	-0.070 (0.023)	0.0020	-0.071 (0.023)	0.0019
Log ₁₀ (VFA)	0.435 (0.041)	1.4×10^{-24}	0.433 (0.041)	2.8×10^{-24}	0.433 (0.041)	2.5×10^{-24}
Log ₁₀ (adiponectin)	-0.233 (0.052)	6.9×10^{-6}	-0.233 (0.052)	7.8×10^{-6}	-0.236 (0.052)	6.2×10^{-6}
HOMA-IR						
SNP	0.051 (0.016)	0.0017	0.048 (0.016)	0.0033	0.049 (0.016)	0.0029
Age	-0.003 (0.001)	0.0012	-0.003 (0.001)	0.0013	-0.003 (0.001)	0.0014
Sex	-0.070 (0.025)	0.0050	-0.070 (0.025)	0.0054	-0.070 (0.025)	0.0048
Log ₁₀ (VFA)	0.502 (0.045)	6.4×10^{-27}	0.500 (0.045)	1.4×10^{-26}	0.500 (0.045)	1.1×10^{-26}
Log ₁₀ (adiponectin)	-0.276 (0.057)	1.3×10^{-6}	-0.276 (0.057)	1.4×10^{-6}	-0.279 (0.057)	1.2×10^{-6}
Total cholesterol						
SNP	-0.729 (1.826)	0.69	-0.934 (1.853)	0.61	-0.693 (1.859)	0.71
Age	0.383 (0.116)	0.0010	0.380 (0.117)	0.0011	0.381 (0.116)	0.0011
Sex	-12.681 (2.832)	8.5×10^{-6}	-12.671 (2.836)	8.9×10^{-6}	-12.595 (2.830)	9.6×10^{-6}
Log ₁₀ (VFA)	26.539 (5.141)	3.0×10^{-7}	26.672 (5.154)	2.8×10^{-7}	26.589 (5.146)	2.9×10^{-7}
Log ₁₀ (adiponectin)	11.912 (6.424)	0.064	12.197 (6.460)	0.059	12.168 (6.462)	0.060
Triglycerides						
SNP	0.030 (0.011)	0.0054	0.025 (0.011)	0.024	0.026 (0.011)	0.020
Age	0.001 (0.001)	0.23	0.001 (0.001)	0.23	0.001 (0.001)	0.24
Sex	0.027 (0.017)	0.10	0.029 (0.017)	0.084	0.028 (0.017)	0.090
Log ₁₀ (VFA)	0.321 (0.030)	7.8×10^{-25}	0.320 (0.030)	1.6×10^{-24}	0.321 (0.030)	1.1×10^{-24}
Log ₁₀ (adiponectin)	-0.258 (0.038)	1.8×10^{-11}	-0.253 (0.038)	5.6×10^{-11}	-0.254 (0.038)	4.7×10^{-11}
HDL-cholesterol						
SNP	-2.444 (0.682)	3.6×10^{-4}	-2.192 (0.693)	0.0016	-2.210 (0.695)	0.0015
Age	0.083 (0.043)	0.055	0.081 (0.044)	0.064	0.082 (0.044)	0.059
Sex	-0.949 (1.058)	0.37	-1.011 (1.060)	0.34	-0.981 (1.058)	0.35
Log ₁₀ (VFA)	-17.432 (1.921)	6.5×10^{-19}	-17.295 (1.927)	1.5×10^{-18}	-17.363 (1.925)	1.0×10^{-18}
Log ₁₀ (adiponectin)	19.665 (2.400)	8.2×10^{-16}	19.617 (2.415)	1.4×10^{-15}	19.599 (2.417)	1.6×10^{-15}
Systolic blood pressure						
SNP	-0.881 (0.872)	0.31	-0.891 (0.886)	0.31	-0.948 (0.888)	0.29
Age	0.403 (0.056)	1.2×10^{-12}	0.397 (0.056)	2.4×10^{-12}	0.402 (0.056)	1.4×10^{-12}
Sex	-3.085 (1.363)	0.024	-3.061 (1.362)	0.025	-3.028 (1.362)	0.026
Log ₁₀ (VFA)	20.949 (2.528)	4.2×10^{-16}	21.143 (2.529)	2.4×10^{-16}	21.007 (2.531)	3.8×10^{-16}
Log ₁₀ (adiponectin)	1.956 (3.053)	0.52	2.218 (3.064)	0.47	2.097 (3.070)	0.49
Diastolic blood pressure						
SNP	0.119 (0.563)	0.83	0.170 (0.572)	0.77	0.160 (0.574)	0.78
Age	0.055 (0.036)	0.13	0.052 (0.036)	0.15	0.055 (0.036)	0.13
Sex	1.659 (0.880)	0.060	1.625 (0.880)	0.065	1.667 (0.879)	0.058
Log ₁₀ (VFA)	14.946 (1.633)	3.5×10^{-19}	15.028 (1.635)	2.5×10^{-19}	14.939 (1.635)	4.0×10^{-19}
Log ₁₀ (adiponectin)	2.218 (1.972)	0.26	2.282 (1.980)	0.25	2.237 (1.983)	0.26

The effect sizes and *P*-values were derived from linear regression analysis. Fasting plasma glucose, insulin, HOMA-IR, and triglycerides were log-transformed for the analysis.

would benefit by considering the *CDH13* genotype and measurement of adiponectin levels.

Decreasing adiponectin levels are thought to be associated with the development of insulin resistance and metabolic disorders^{3, 5-8}. Adiponectin-inducing alleles of *CDH13* SNPs were shown to cause the deterioration of several metabolic syndrome traits. These effects were independent of visceral fat accumulation and adiponectin levels, which affect metabolic syndrome traits, consistent with a previous study by Gao *et al.*²¹. The path diagram showed that *CDH13* polymorphisms could exacerbate metabolic syndrome, independent of their effect on increasing adiponectin levels. *CDH13* encodes T-cadherin, a receptor for adiponectin¹⁸, and *CDH13*-deficient mice show a phenotype similar to that of adiponectin-deficient mice, without response to adiponectin supplementation²⁷. Since rs3865188 exists 9 kb upstream of the transcription start site of the *CDH13* gene and rs4783244 and rs12051272 in intron 1, these SNPs may affect transcriptional activity. These data suggest that individuals with adiponectin-inducing alleles of *CDH13* have higher adiponectin levels; however, they have increased symptoms of metabolic disorders because of the lower expression of *CDH13*, an adiponectin receptor.

One of the mechanism through which *CDH13* SNPs may affect the expression of the *CDH13* gene was reported by Putku *et al.*²⁸, who showed that some of SNPs around the CpG island within the regulatory region of the *CDH13* gene affect the methylation levels of the gene region. Adiponectin-inducing alleles of SNPs (rs8060301 and rs12444338) were related to hypermethylation within the CpG island, which would reduce the expression of *CDH13*. The three SNPs examined in this study (i.e., rs3865188, rs4783244, and rs12051272) were located near rs8060301 and rs12444338 in linkage disequilibrium (LD, $r^2 > 0.9$; **Supplementary Fig. 1**). Therefore, these three SNPs in the *CDH13* gene are expected to affect the methylation and expression of *CDH13*, resulting in the occurrence of metabolic disorders, despite higher adiponectin levels. This phenomenon may be considered adiponectin resistance. T-cadherin has been identified as an adiponectin-binding protein with preference for high-molecular-weight (HMW) adiponectin multimers. However, in this study, we did not measure HMW adiponectin. Because both total and HMW adiponectin are related to metabolic syndrome^{29, 30}, further studies are needed to investigate the relationship between *CDH13* SNPs and metabolic syndrome including HMW adiponectin levels.

Adiponectin levels are affected by various factors other than age, sex, VFA and *CDH13* SNPs. Varia-

tions in the adiponectin, C1Q and collagen domain containing (*ADIPOQ*) gene are also known to determine adiponectin levels and affect metabolic syndrome³¹. We have recently reported that some *ADIPOQ* SNPs are associated with adiponectin levels and insulin resistance³². We found that each *ADIPOQ* (rs10937273 or rs1648707) and *CDH13* SNP was independently associated with adiponectin levels. SEM analysis showed that each *ADIPOQ* SNP affected adiponectin levels, but not risk clustering.

Adiponectin levels have been shown to be increased due to reduced renal function³³⁻³⁵ and decreased by cigarette smoking³⁶. Reduced renal function would decrease the clearance of adiponectin, resulting in an increase in circulating adiponectin levels, even in the healthy subjects. Further studies including renal function and cigarette smoking would support our hypothesis regarding the presence of an adiponectin-resistant state.

Conclusion

We found that three SNPs (rs3865188, rs4783244, and rs12051272) in the *CDH13* gene were associated with increased adiponectin levels; however, these exacerbated metabolic syndrome traits, independent of visceral fat accumulation. These SNPs could affect adiponectin levels and metabolic syndrome traits through the adiponectin-resistant state. Further studies are necessary to clarify the effects of *CDH13* SNPs on the adiponectin-resistant state.

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Supplementary Fig. 1. LD map of SNPs in *CDH13*