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Adiponectin Receptor gene Polymorphisms are Associated with Kidney Function in Elderly Japanese Populations

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Aim: Adiponectin exhibits its biological effects through adiponectin receptors (AdipoR1 and AdipoR2), which are distributed in the kidneys, and activation of those receptors could prevent or ameliorate diabetic nephropathy. This study aimed to evaluate the associations between *AdipoR* single nucleotide polymorphisms (SNPs) and kidney function in an elderly Japanese population.

Methods: A total of 271 elderly Japanese volunteers underwent anthropometric and laboratory tests (cystatin C-based eGFR and total and high molecular weight adiponectin levels at baseline and a follow-up visit). Genotype data were obtained for the selected 7 and 5 *AdipoR1* and *AdipoR2* SNPs, respectively.

Results: In a cross-sectional analysis at baseline, we found a significant association between the *AdipoR2* SNP rs12230440 and kidney function; eGFRcys tended to increase as the number of carriers of T alleles increased after adjustment for covariates and Bonferroni correction, although the association of the SNP and annual eGFR decline could not be identified in the longitudinal data. Regarding the variants rs16850797, rs11061925, and rs10773983, each of the allele G, allele C, and allele G showed nominally significant associations with higher eGFRcys. However, this failed to reach significance after Bonferroni correction.

Conclusion: Here, an *AdipoR2* SNP was associated with kidney function, suggesting that the effects of this polymorphism on adiponectin receptor may affect kidney function in the elderly Japanese population.

Key words: Adiponectin receptor, Single nucleotide polymorphism, Kidney function

Introduction

Chronic kidney disease (CKD) is prevalent in approximately 8−16% of the world's population[1)]. The proportion of individuals with CKD increases markedly with age, and elderly adults are more vulnerable to the metabolic and hormonal disturbances associated with kidney function decline[2)]. In aged populations, decreased kidney function has been suggested to be a contributing factor to atherosclerosis, which causes cardiovascular and cerebrovascular disease, and leads to high incidents of mortality[3-8)]. Furthermore, in older adults, CKD has been associated with non-cardiovascular complications such as infection, cognitive decline, poor physical functioning, and frailty[9-14)]. Therefore, clarification of the pathogenesis of kidney function decline, which is related to both genes and the environment, is very important[15)]. In the genetic aspect, genome-wide association studies have identified more than 30 genetic variants influencing interindividual variation in kidney function by identifying loci that potentially influence nephrogenesis, podocyte function, angiogenesis, solute transport, and metabolic functions of the kidney[16-19)].

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Adiponectin is a major adipokine secreted by adipose tissue that plays an important role in regulating glucose and fat metabolism by enhancing insulin sensitivity and decreasing free fatty acid production. Adiponectin exhibits its biological effects through adiponectin receptors (AdipoR), including AdipoR1 and AdipoR2, both of which contain 7 transmembrane domains and are topologically distinct from the G protein-coupled receptor[20]. AdipoR1 mediates increases in 5′-adenosine monophosphate-activated protein kinase, and AdipoR2 activates peroxisome proliferator-activated receptor (PPAR)-α. AdipoR1 is widely expressed in muscle tissue, whereas AdipoR2 is expressed predominantly in the liver[21, 22]. In addition, other studies have shown that AdipoR1 and AdipoR2 are distributed in the kidneys, and their activation could prevent and ameliorate diabetic nephropathy, especially type 2 diabetes mellitus (T2DM)[23, 24]. Indeed, rats with CKD had increased renal expression of AdipoR1 and AdipoR2, which indicates that renal injury may cause compensatory upregulation of AdipoR1 and AdipoR2 in the kidneys to abate further injury. These renoprotective effects may partly be explained by ameliorating glomerular endothelial cell and podocyte injury by activating the AMP-activated protein kinase/PPAR-α pathway through AdipoR. Recent studies have demonstrated that *AdipoR* polymorphisms are associated with coronary artery disease, intima media thickness, and endothelial dysfunction[25]. Thus, we hypothesized that *AdipoR* polymorphism might affect kidney function via AdipoR activation signaling.

However, the association of *AdipoR1* and *AdipoR2* polymorphisms with kidney function in the elderly general population has not been fully understood. In this study, we evaluated the association of polymorphisms of *AdipoR1* and *AdipoR2* genes—7 single nucleotide polymorphisms (SNPs) for *AdipoR1* (rs7539542, rs1342387, rs16850797, rs12045862, rs16850799, rs2275737, and rs1539355) and 5 SNPs for *AdipoR2* (rs11061925, rs10773983, rs10773986, rs10773988, and rs12230440)—with kidney function, as measured by cystatin C, in community-dwelling elderly adults.

Methods

Study Design

This is a prospective observational study of community-dwelling elderly adults aimed to evaluate the hematologic changes accompanying increasing age. All participants provided written informed consent to participate during their first visit and the study protocol was approved by the Ethics Committee of Nihon University School of Medicine in accordance with the Declaration of Helsinki. This study was conducted from 2004 to 2015 in Ogano-machi, a town of approximately 12,000 residents located in Saitama Prefecture, Japan. Volunteers were recruited based on the method outlined in a previous report[26]. By 2015, 1034 residents had participated in the study. Of these participants, subjects who were followed for more than two times (between 2004 and 2015) were enrolled. Subjects who did not undergo baseline or follow-up blood sampling were excluded. Frozen sera, collected at two different times from a single subject and stored at −80℃, were used to measure the serum total adiponectin, glycated albumin, albumin, and cystatin C. In cases where >2 samples were drawn from a subject, we used the sample collected at the time point furthest away from the baseline.

Data Collection

As in our previous study[26], participants were asked to answer a standard questionnaire and were measured for height, body weight, body mass index (BMI), waist circumference, and blood pressure. Non-fasting blood samples were drawn from all participants using the antecubital vein, and laboratory parameters measured at the annual evaluations included serum high molecular weight (HMW) adiponectin, high-density lipoprotein (HDL) cholesterol levels, total cholesterol levels, and triglyceride levels. HMW adiponectin levels were determined by the chemiluminescent enzyme immunoassay using a Lumipulse® *f* analyzer (Fujirebio, Tokyo, Japan). The intra-assay and inter-assay coefficients of variation were 5.2−6.9% and 2.8−4.5%, respectively. Direct measurement of HDL cholesterol was conducted at a central laboratory (SRL Inc., Tokyo, Japan). In addition to the above-mentioned measurement of parameters, in 2016 we thawed the baseline sera samples that had been stored at −70− −80℃ and measured additional parameters, including serum cystatin C, total adiponectin, creatinine, albumin, and GA levels at baseline, and compared them to the follow-up values. Serum cystatin C was measured using a colloidal gold particle-enhanced colorimetric immunoassay (Nescauto GC Cystatin C, Alfresa Pharma, Osaka, Japan). The coefficient of variation for the cystatin C assay was ≤10% during the testing period, and the analytical measurement range for cystatin C was 0.20−8.00 mg/L. Total adiponectin was measured by a latex turbidimetric immunoassay using a Human Adiponectin Latex Kit (LSI Medience Corporation, Tokyo, Japan). The coefficient of variation for total adiponectin was ≤10% during the testing period, and the analytical measurement range for total adiponectin was 0.5−25 μg/mL. Albumin was determined by a bromocresol purple dye-binding assay (PureAuto S ALB; Kainos, Tokyo, Japan). GA was measured using the LU-

CICA GA-L kit (Asahi Kasei Pharma Corporation, Tokyo, Japan). Mean arterial pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure. Because serum creatinine level is affected by muscle mass, we chose to use cystatin C to calculate eGFR. eGFR by cystatin C (eGFRcys) was calculated using the following equations: eGFRcys in male subjects = $(104 \times \text{cystatin C}^{-1.094} \times 0.996^{\text{Age}}) - 8$; eGFRcys in female subjects = $(104 \times \text{cystatin C}^{-1.019} \times 0.996^{\text{Age}} \times 0.929) - 8$[27].

Selection of SNPs

To investigate the genetic variability in each gene, we used a tag SNP approach. This tagging approach was applied to the entire set of common genetic variants in the *AdipoR1* and *AdipoR2* genes with a minor allele frequency (MAF) of ≥ 10% in the Japanese population (JPT) and a minimum r^2 threshold of 0.8. The tagging SNPs were selected using the NIEHS LD TAG SNP Selection database (https://snpinfo.niehs.nih.gov/snpinfo/snptag.html). This process resulted in the selection of 9 and 11 tagging SNPs for the *AdipoR1* and *AdipoR2* genes, respectively. We chose genotype rs7539542 (UTR variant 3 prime), rs1342387 (intron variant), rs16850797 (intron variant), rs12045862 (intron variant), rs16850799 (intron variant), rs2275737 (intron variant), rs1539355 (intron variant), for *AdipoR1* and rs10773983 (intron variant), rs10773988 (intron variant), rs12230440 (intron variant), for *AdipoR2* after careful review of the literature and their MAFs in the Japanese population[28-34]. In addition, although they were not tag SNPs, rs11061925 (intron variant) and rs10773986 (intron variant) were evaluated because rs11061925 was shown the relation between the SNP and the metabolic parameter[35], and rs10773986 had high MAF in the Japanese population.

Single-Nucleotide Polymorphism Analysis

Genomic DNA was extracted from peripheral blood leukocytes by the phenol-chloroform method[36]. Genotyping was performed using the TaqMan® SNP genotyping assay (Applied Biosystems, Foster City, CA, USA)[37].

TaqMan® SNP genotyping assays used were C_30041594_10 (rs7539542), C_37350_10 (rs1342387), C_32757249_10 (rs16850797), C_30887083_10 (rs12045862), C_32757247_10 (rs16850799), C_15879522_10 (rs2275737), C_11457922_10 (rs1539355), C_26747669_10 (rs11061925), C_2633801_20 (rs10773983), C_334325_10 (rs10773986), C_2636032_20 (rs10773988), and C_2636031_20 (rs12230440). PCR amplification was performed with a 5-μL final reaction volume, containing 2.5 μL TaqMan® universal master mix, 2 ng DNA, 2.375 μL ultrapure water, 0.079 μL Tris-EDTA buffer (1×), and 0.046 μL TaqMan® SNP genotyping assay mix (40×) containing primers (final concentration, 331.2 nmol/L) and probes (final concentration, 73.6 nmol/L). Thermal cycling was performed using the GeneAmp 7700 system (Applied Biosystems, Foster City, CA, USA) under the following conditions: 50 cycles of 50℃ for 2 min and 95℃ for 10 min, and 50 cycles of 95℃ for 15 s and 60℃ for 1 min. Each 96-well plate contained 80 DNA samples of unknown genotype and four reaction mixtures containing reagents without DNA as controls, which were a necessary part of the 7700 signal processing system as outlined in the TaqMan® Allelic Discrimination Getting Started Guide (Applied Biosystems, Foster City, CA, USA). The plates were read on the 7500 Real-time PCR System (version 1.4; Applied Biosystems, Foster City, CA, USA). Genotypes were determined visually based on the dye component fluorescent emission data depicted on X-Y scatter plots by the sequence detection system software. Genotypes were also determined automatically by the software's signal processing algorithms. The results of each scoring method were saved in separate output files for subsequent comparisons[38].

SNP Quality Control

SNP-level quality control metrics were applied prior to the analyses. All genotyping SNPs showed: a call rate ≥ 95%, MAF ≥ 10%, Hardy–Weinberg equilibrium (HWE) $p > 0.05$. **Supplementary Table 1** shows the characteristics, MAF, and HWE *P*-values for the *AdipoR1* and *AdipoR2* SNPs.

Statistical Analyses

SPSS version 24 (SPSS Inc, Chicago, IL) was used for descriptive statistical analysis. Participant characteristics were summarized by the medians (interquartile range: 25-75 percentile) for continuous variables and percentages for categorical variables. The Mann–Whitney *U* test was used to compare the two groups. Comparisons for categorized variables were tested with the Fisher exact test. Associations between *AdipoR1* and *AdipoR2* SNPs and the continuous variables (eGFRcys, total adiponectin, and HMW adiponectin) were tested using linear regression of additive (2 vs. 1 vs. 0 risk alleles) models of inheritance respectively adjusted for age, gender, BMI, MAP, total cholesterol, HDL cholesterol, GA, antidiabetic medications, and antihypertensive medications. In addition, the association of annual eGFRcys decline and the SNP which was significantly associated with baseline eGFRcys was tested via a logistic regression analysis of recessive (2 vs. 0/1 risk alleles) and dominant (2/1 vs. 0 risk alleles) models of inheritance and via linear regression of additive (2 vs. 1 vs. 0 risk alleles) models of inheritance adjusted for

Table 1. Baseline characteristics of the study participants

	Total (n=271)	eGFRcys ≥60 mL/min/1.73 m^2 (n=216)	eGFRcys <60 mL/min/1.73 m^2 (n=55)	P value
Age, years	73.0 (69.0-77.0)	72.0 (68.0-76.9)	76.0 (73.0-80.5)	<0.0001
Sex, male %	28.4 (77/271)	26.9 (58/216)	34.5 (19/55)	0.26
Body mass index, kg/m^2	23.0 (21.2-24.7)	22.9 (21.2-24.7)	23.4 (21.2-25.5)	0.38
Current smoking status, %	12.5 (34/271)	12.0 (26/216)	14.5 (8/55)	0.62
SBP, mmHg (n=268)	143.0 (131.5-154.0)	143.0 (130.0-154.0)	143.0 (136.0-158.0)	0.21
DBP, mmHg (n=270)	77.0 (70.0-84.0)	76.0 (70.0-84.0)	78.0 (72.0-82.0)	0.30
MAP, mmHg (n=269)	98.3 (91.8-107.8)	97.7 (90.7-108.0)	99.3 (94.3-107.7)	0.17
GA, %	15.0 (14.2-16.0)	15.1 (14.2-16.0)	15.0 (14.1-16.5)	0.83
Total cholesterol, mg/dL	202.0 (180.5-227.0)	205.0 (183.0-227.0)	187.0 (170.3-224.8)	0.037
HDL cholesterol, mg/dL	55.5 (45.0-64.0)	57.0 (46.0-66.0)	49.0 (38.5-57.0)	<0.0001
LDL cholesterol, mg/dL	116.8 (97.4-139.2)	117.8 (99.6-138.4)	113.8 (90.1-144.5)	0.73
TG, mg/dL	130.5 (96.0-177.0)	130.0 (96.0-174.0)	135.0 (101.0-189.0)	0.36
Total adiponectin, ng/mL	11.8 (8.7-17.8)	12.0 (8.6-17.4)	10.7 (8.7-18.9)	0.83
HMW adiponectin, ng/mL	7.0 (4.4-10.7)	7.1 (4.4-10.9)	6.6 (4.3-9.8)	0.29
Cystatin C, mg/L	0.93 (0.83-1.05)	0.89 (0.81-0.98)	1.22 (1.13-1.38)	<0.0001
eGFRcys, mL/min/1.73 m^2	70.5 (61.6-82.1)	74.6 (67.3-84.6)	50.9 (45.5-56.1)	<0.0001
Antihypertensive medication, %	40.0 (108/271)	38.4 (83/216)	45.5 (25/55)	0.34
Antidiabetic medication, %	7.8 (20/271)	6.9 (15/216)	9.1 (5/55)	0.59

Data are shown as medians (25th and 75th percentile). SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; GA, glycated albumin; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; TG, triglyceride; HMW adiponectin, high molecular weight adiponectin; eGFRcys, cystatin C-based estimated glomerular filtration rate.

age, gender, BMI, MAP, total cholesterol, HDL cholesterol, GA, antidiabetic medications, antihypertensive medications, and baseline eGFRcys. A Bonferroni correction was calculated based on the number of individual SNPs examined (0.05/12). Therefore, a P-value threshold of 0.0042 was used to determine the Bonferroni-corrected statistical significance for AdipoR1 and AdipoR2 SNPs. Total adiponectin level, HMW adiponectin level, MAP, HDL cholesterol, and GA were log-transformed to obtain better approximations of the normal distribution prior to analysis.

The haplotype-based analyses were conducted using SNPAlyze version 3.2. (DYNACOM Co., Ltd., Chiba, Japan). Haplotypes with estimated frequency of <5% were excluded from the analyses. To conduct the haplotype analysis that included common SNPs significantly or nominally associated with the study outcome variables, the P-value threshold of 0.0042 was accepted to determine the Bonferroni-corrected statistical significance (0.05/12). Multiple linear regression models were used to test the associations between the haplotypes and the study outcome variables. All the models were adjusted for age, gender, BMI, MAP, total cholesterol, HDL cholesterol, GA, antihypertensive medications, and antidiabetic medications.

Results

Baseline Characteristics of the Study Participants

The baseline characteristics of the study participants according to eGFRcys are shown in **Table 1**. Of the 271 participants, 216 had an eGFRcys ≥60; the rest had values <60. The participants' ages were significantly higher in the group with eGFRcys <60 than in that with eGFRcys ≥60 (76.0 vs. 72.0 years, P<0.0001). Total cholesterol and HDL cholesterol were significantly higher in the eGFRcys ≥60 group than in the eGFRcys <60 group (205.0 mg/dL vs. 187.0 mg/dL, P=0.037; 57.0 mg/dL vs. 49.0 mg/dL, P<0.001). There were no statistically significant differences in sex, smoking status, BMI, SBP, DBP, MAP, GA, LDL cholesterol, TG, total adiponectin, HMW adiponectin, antihypertensive medications, and antidiabetic medications between the two groups.

Associations of *AdipoR1* and *AdipoR2* gene Polymorphisms with Kidney Function, Total Adiponectin, and HMW Adiponectin Levels

The associations between *AdipoR* gene SNPs and kidney function, total adiponectin, and HMW adiponectin levels are presented in **Table 2**. The variant rs 12230440 was found to be significantly associated with

Table 2. Association of ADIPOR1 and ADIPOR2 SNPs with eGFRcys, total adiponectin, and HMW adiponectin

SNPs		eGFRcys			Log Total adiponectin			Log HMW adiponectin		
rs	Alleles	β (SE)	*P* value	*P* value (Bonferroni Corr.)	β (SE)	*P* value	*P* value (Bonferroni Corr.)	β (SE)	*P* value	*P* value (Bonferroni Corr.)
ADIPOR1										
rs7539542	G/C	−1.12 (1.38)	0.42	1	0.023 (0.022)	0.28	1	−0.0024 (0.030)	0.94	1
rs1342387	T/C	−1.73 (1.10)	0.12	1	0.024 (0.017)	0.16	1	−0.0017 (0.024)	0.95	1
rs16850797	G/C	3.05 (1.20)	0.011	0.13	−0.044 (0.018)	0.018	0.22	−0.035 (0.026)	0.18	1
rs12045862	T/C	−1.12 (1.16)	0.34	1	0.018 (0.018)	0.33	1	0.012 (0.025)	0.65	1
rs16850799	G/A	1.74 (1.17)	0.14	1	−0.043 (0.018)	0.019	0.23	−0.045 (0.025)	0.079	0.95
rs2275737	C/A	−0.14 (1.30)	0.92	1	0.016 (0.020)	0.43	1	0.026 (0.028)	0.36	1
rs1539355	A/G	−2.34 (1.60)	0.14	1	0.0031 (0.025)	0.90	1	−0.025 (0.035)	0.47	1
ADIPOR2										
rs11061925	C/T	2.34 (1.12)	0.038	0.46	−0.0075 (0.018)	0.67	1	−0.0032 (0.025)	0.90	1
rs10773983	G/A	3.05 (1.10)	0.0060	0.072	−0.0064 (0.017)	0.71	1	0.016 (0.024)	0.52	1
rs10773986	A/G	1.35 (1.11)	0.23	1	0.0054 (0.17)	0.75	1	−0.0063 (0.024)	0.80	1
rs10773988	A/G	−1.70 (1.11)	0.13	1	−0.013 (0.017)	0.45	1	−0.013 (0.024)	0.58	1
rs12230440	T/G	3.57 (1.19)	0.0028	0.034	−0.14 (0.19)	0.44	1	−0.0051 (0.026)	0.85	1

Alleles listed as major/minor allele; Multiple linear regression model for eGFRcys adjusted for age, gender, body mass index, mean arterial pressure, total cholesterol, high-density lipoprotein cholesterol, glycated albumin, antihypertensive medications, and antidiabetic medications. Multiple linear regression model for total and HMW adiponectin adjusted for age, gender, body mass index, total cholesterol, high-density lipoprotein cholesterol, and glycated albumin. ADIPOR, adiponectin receptor; SNP, single nucleotide polymorphism; eGFRcys, cystatin-based estimated glomerular filtration rate; HMW adiponectin, high-molecular-weight adiponectin; SE, standard error; Bonferroni Corr, Bonferroni Correction.

eGFRcys (analysis of recessive and dominant models for rs12230440 is shown in **Supplementary Table 2**). The analysis showed that eGFRcys tended to increase as the number of carriers of T alleles increased after adjustments for age, gender, BMI, MAP, GA, total-cholesterol, HDL cholesterol, antihypertensive medication, and antidiabetic medication and after Bonferroni correction ($\beta = 3.57$, corrected $P = 0.034$). In the case of the variants rs16850797, rs11061925, and rs10773983, each of the G allele, allele C, and allele G showed a nominally significant association with higher eGFRcys as well. However, this failed to reach the significance threshold after Bonferroni correction (corrected $P = 0.13$, 0.46, and 0.072).

In the prospective analysis, for which the average follow-up period was 4.59 years, median annual eGFRcys decline was −1.7 ml/min/1.73 m^2. There was no significant association between rs12230440 variants and annual eGFRcys decline after multivariable adjustments when analyzed using recessive ($P = 0.39$), dominant ($P = 0.28$), and additive ($P = 0.16$) models of inheritance (**Table 3**).

We could not detect significant associations between the *AdipoR1* and *AdipoR2* variants and HMW adiponectin levels. On the other hand, total adiponectin levels tended to significantly decrease as the number of major alleles G of the SNPs rs16850797 and rs16750799 increased. However, this failed to reach the significance threshold after Bonferroni correction (corrected $P = 0.22$ and 0.23).

Associations of Common Haplotypes of *AdipoR2* with Kidney Function

To study the combined effect of *AdipoR2* SNPs in the present study, the haplotype analysis only included combinations of those *AdipoR2* variants that were significantly or nominally associated with baseline eGFRcys (i.e., rs11061925, rs10773983, and rs12230440). The haplotype analysis predicted three common haplotypes (frequency >5%). The most probable haplotype (CGT) had an estimated global frequency of 61%. As shown in **Table 4**, the haplotype of TAG had significant association with eGFRcys, which reached the significance threshold after Bonferroni correction and adjustment for all the covariates ($\beta = -3.83$, corrected $P = 0.019$).

Discussion

In this study, we analyzed the associations of genetic variants of *AdipoR1* and *AdipoR2* genes and haplotypes of the *AdipoR2* gene with kidney function and adiponectin level in elderly Japanese populations. One of the main findings was the significant association

Table 3. Association of rapid eGFRcys decline and SNP of rs12230440

	β (SE)	*P* value by model of inheritance		
		Recessive	Dominant	Additive
Continuous outcome				
Annual eGFRcys decline	0.27 (0.20)	0.39	0.28	0.16

Multivariable models adjust for age, gender, body mass index, mean arterial pressure, total cholesterol, high-density lipoprotein cholesterol, glycated albumin, antihypertensive medications, antidiabetic medications, and baseline eGFRcys. eGFRcys, cystatin C-based estimated glomerular filtration rate; SNP, single nucleotide polymorphism; SE, standard error.

Table 4. Association of the common haplotype combinations in the ADIPOR2 gene with eGFRcys

Haplotype	F	eGFRcys		
		β (SE)	*P* value	*P* value (Bonferroni Corr.)
CGT	0.61	2.06 (1.08)	0.059	0.71
TAG	0.27	−3.83 (1.12)	0.0016	0.019
CAT	0.058	0.024 (2.41)	0.99	1

The results of the association are listed as the Beta (standard error) (β (SE)) with the corresponding raw *P*-value. β coefficients represent the change in eGFRcys of each additional risk haplotype. Model adjusted for age, gender, body mass index, mean arterial pressure, total cholesterol, high-density lipoprotein cholesterol, glycated albumin, antihypertensive medications, and antidiabetic medications. ADIPOR, adiponectin receptor; eGFRcys, cystatin-based estimated glomerular filtration rate; F, haplotype frequency; SE, standard error; Bonferroni Corr, Bonferroni Correction.

between the T allele of the rs12230440 SNP with higher eGFRcys, which remained significant even after the adjustment for covariates and Bonferroni correction. Furthermore, we found that carriers of the G allele of rs16850797 and rs10773983, and the C allele of rs11061925, showed a nominally significant association with higher eGFRcys. To our knowledge, this study is the first to analyze the association of *AdipoR1* and *AdipoR2* with eGFRcys in the elderly general population.

To date, numerous studies have been published providing details on the association between adiponectin and kidney function. With these studies comes the knowledge that patients with CKD have significantly increased levels of serum adiponectin[39-41]; some studies have even proposed that elevated levels of serum adiponectin serve as a prognostic marker in CKD progression[42]. Others advocate the notion that adiponectin offers kidney protection under CKD conditions[43-45]. Dissecting the mechanisms of adiponectin at the fine-scale level, we now understand that this unique adipokine exerts its effects via interaction with specific adiponectin receptors, AdipoR1 and AdipoR2[20, 46].

Although, this study is the first to analyze the association of *AdipoR* SNPs with kidney function, there are some reports detailing the relationship between *AdipoR* SNPs and CVD risk. Recently, a cross-sectional study population revealed that an intronic variant, rs767870, is associated with coronary artery disease, intima-media thickness, and endothelial dysfunction[25]. In addition to 13 other *AdipoR2* SNPs, this variant is also associated with fasting plasma triglyceride concentrations in Mexican[47] and Finnish[48] subjects. Other reports, studying the Finnish population, have demonstrated that *AdipoR2* SNPs in rs11061937 and rs1058322 were associated with CVD risk[34]. Furthermore, patients with an inherent homozygosity for the rare alleles rs11061946 and rs11061973 displayed an increased risk of converting from impaired glucose tolerance to T2DM.

One of the causes of the association between *AdipoR* SNPs and CVD risk might be described by the influence that *AdipoR* SNPs assume over the interaction between monocytes/macrophages and adiponectin, which is of critical importance in the anti-atherosclerotic protection offered by adiponectin[47]. Chronic inflammation is of key importance in the process of renal interstitial fibrosis via monocytes/macrophages[49-51]. Taking into consideration that rs11061937 and rs11061946 are SNPs tagged to rs12230440, which has been demonstrated in the present study to be associated with kidney function, one can hypothesize that the *AdipoR2* SNP affects—albeit to an undetermined degree—fibrotic replacement in the renal interstitium under chronic in-

flammatory conditions. The specific mechanism is unclear, but the susceptibility of AdipoR to adiponectin or the expression of AdipoR might be affected by this intronic SNP. Indeed, AdipoR1 and R2 double-knockout mice diminished adiponectin-binding effects, which are insulin resistance and impaired glucose tolerance, increased inflammation, and oxidative stress[52, 53]. These effects imply the possibility of anti-inflammatory effects on the kidney tissue via AdipoR. The specific mechanisms underlying the regulation of AdipoR1 and AdipoR2 remain an elusive subject; however, altered expression of AdipoR1 and AdipoR2 in various forms of tissue have been reported in numerous pathological conditions[54-57]. These facts illustrate the possibility of AdipoR regulation being coordinated in an organ-specific manner and that genetic factors are one of the most crucial factors contributing to the expression and regulation of this gene. Consistent with this theory, Halvatsiotis *et al.* observed that the *AdipoR2* variant rs767 870 altered AdipoR2 protein expression levels in peripheral monocytes[25].

Although the findings of this particular study are unique and offer insight into the association of *AdipoR1* and *AdipoR2* with eGFRcys, it does have several limitations. First, our study was limited by the relatively small sample population adopted for genetic association studies. This may weaken the discriminatory statistical power to identify true associations of genetic variants and clinical characteristics. Secondly, our study's participants were mainly restricted to the elderly population. Hence, it is difficult to apply our results to the young or middle aged Japanese populations. The third limitation lies in the fact that urinary protein levels were not evaluated. Urinary protein is a potentially key factor in kidney function decline. Lastly, we could not gather information pertaining to the use of cholesterol-lowering medications taken by the participants in this study. This is an important limitation because the use of such medications may alter serum adiponectin levels.

Conclusion

In this study, conducted in an elderly healthy Japanese cohort, we identified significant associations between an *AdipoR2* SNP and eGFRcys that suggest a potential effect of *AdipoR* gene variants on kidney function. These findings contribute to the understanding of the mechanism by which adiponectin exerts its effects on kidney function via AdipoR. However, these associations need further clarification of AdipoR expression at both the genetic and protein levels. Additional studies that focus on the adiponectin pathway and gene-environment interactions in multi-ethnic subjects, will aid us in obtaining a deeper understanding of the biological differences underlying the *AdipoR* gene and kidney function.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1) Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FGR, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez- Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Wein-

stock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, and Memish ZA: Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 2012; 380: 2095-2128. DOI: 10.1053/j.ajkd.2009.09.035

2) Stevens LA, Li S, Wang C, Huang C, Becker BN, Bomback AS, Brown WW, Burrows NR, Jurkovitz CT, McFarlane SI, Norris KC, Shlipak M, Whaley-Connell AT, Chen SC, and Bakris GL, and McCullough PA: Prevalence of CKD and comorbid illness in elderly patients in the United States: results from the Kidney Early Evaluation Program (KEEP). Am J Kidney Dis, 2010; 55(3 Suppl 2): S23-33
3) Lindner A, Charra B, Sherrard DJ, and Scribner BH. Accelerated atherosclerosis in prolonged maintenance hemodialysis. N Engl J Med, 1974; 290: 697-701
4) Bittencourt MS, Hulten EA, Ghoshhajra B, Abbara S, Murthy VL, Divakaran S, Nasir K, Gowdak LH, Riella LV, Chiumiento M, Hoffmann U, Di Carli MF, and Blankstein R. Incremental prognostic value of kidney function decline over coronary artery disease for cardiovascular event prediction after coronary computed tomography. Kidney Int, 2015; 88: 152-159
5) Drueeke TB and Massy ZA. Atherosclerosis in CKD: differences from the general population. Nat Rev Nephrol. 2010; 6: 723-735
6) Foley RN, Parfrey PS, and Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis, 1998; 32: S112-S119
7) Nakayama M, Metoki H, Terawaki H, Ohkubo T, Kikuya M, Sato T, Nakayama K, Asayama K, Inoue R, Hashimoto J, Totsune K, Hoshi H, Ito S, and Imai Y. Kidney dysfunction as a risk factor for first symptomatic stroke events in a general Japanese population--the Ohasama study. Nephrol Dial Transplant, 2007; 22: 1910-1905
8) Synhaeve NE, van Alebeek ME, Arntz RM, Maaijwee NA, Rutten-Jacobs LC, Schoonderwaldt HC, de Kort PL, van der Vlugt MJ, Van Dijk EJ, Wetzels JF, and de Leeuw FE: Kidney dysfunction increases mortality and incident events after young stroke: the FUTURE study. Cerebrovasc Dis, 2016; 42: 224-231
9) Dalrymple LS, Katz R, Kestenbaum B, deBoerIH, Fried L, Sarnak MJ, and Shlipak MG: The risk of infection-related hospitalization with decreased kidney function. Am J Kidney Dis, 2012; 59: 356-363
10) Bai K, Pan Y, Lu F, Zhao Y, Wang J, and Zhang L: Kidney function and cognitive decline in an oldest-old Chinese population. Clin Interv Aging, 2017; 12: 1049-1054
11) Fried LF, Katz R, Sarnak MJ, Shlipak MG, Chaves PH, Jenny NS, Stehman-Breen C, Gillen D, Bleyer AJ, Hirsch C, Siscovick D, and Newman AB: Kidney function as a predictor of non- cardiovascular mortality. J Am Soc Nephrol, 2005; 16: 3728-3735
12) Fried LF, Lee JS, Shlipak M, Chertow GM, Green C, Ding J, Harris T, and Newman AB: Chronic kidney disease and functional limitation in older people: health, aging and body composition study. J Am Geriatr Soc, 2006; 54: 750-756
13) Sarnak MJ, Katz R, Fried LF, Siscovick D, Kestenbaum B, Seliger S, Rifkin D, Tracy R, Newman AB, and Shlipak MG: Cardiovascular Health Study: Cystatin C and aging success. Arch Intern Med, 2008; 168: 147-153
14) Dalrymple LS, Katz R, Rifkin DE, Siscovick D, Newman AB, Fried LF, Sarnak MJ, Odden MC, and Shlipak MG: Kidney function and prevalent and incident frailty. Clin J Am Soc Nephrol, 2013; 8: 2091-2099
15) Rao F, Wessel J, Wen G, Zhang L, Rana BK, Kennedy BP, Greenwood TA, Salem RM, Chen Y, Khandrika S, and Hamilton BA: Renal albumin excretion: twin studies identify influences of heredity, environment, and adrenergic pathway polymorphism. Hypertension, 2007; 49: 1015-1031
16) Chambers JC, Zhang W, Lord GM, Van der Harst P, Lawlor DA, Sehmi JS, Gale DP, Wass MN, Ahmadi KR, Bakker SJ, and Beckmann J: Genetic loci influencing kidney function and chronic kidney disease. Nat Genet, 2010; 42: 373-375
17) Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, Parsa A, Gao X, Yang Q, Smith AV, and O'Connell JR: New loci associated with kidney function and chronic kidney disease. Nat Genet, 2010; 42: 376-384
18) Köttgen A, Glazer NL, Dehghan A, Hwang SJ, Katz R, Li M, Yang Q, Gudnason V, Launer LJ, Harris TB, and Smith AV: Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet, 2009; 41: 712-717
19) Pattaro C, Köttgen A, Teumer A, Garnaas M, Böger CA, Fuchsberger C, Olden M, Chen MH, Tin A, Taliun D, Li M: Genome-wide association and functional follow-up reveals new loci for kidney function. PLoS Genet. 2012; 8: e1002584
20) Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, and Tobe K: Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest, 2006; 116: 1784-1792
21) Cammisotto PG and Bendayan M. Adiponectin stimulates phosphorylation of AMP-activated protein kinase alpha in renal glomeruli. J Mol Histol, 2008; 39: 579-584
22) Wang Y, Zhou M, Lam KS, and Xu A: Protective roles of adiponectin in obesity- related fatty liver diseases: mechanisms and therapeutic implications. Arq Bras Endocrinol Metabol, 2009; 53: 201-212
23) Yu Y, Bao BJ, Fan YP, Shi L, and Li SQ: Changes of adiponectin and its receptors in rats following chronic renal failure. Renal Failure, 2013; 36: 92-97
24) Christou GA and Kiortsis DN: The role of adiponectin in renal physiology and development of albuminuria. J Endocrinol, 2014; 221: R49-R61
25) Halvatsiotis I, Tsiotra PC, Ikonomidis I, Kollias A, Mitrou P, Maratou E, Boutati E, Lekakis J, Dimitriadis G, Economopoulos T, Kremastinos DT, and Raptis SA: Genetic variation in the adiponectin receptor 2 (ADIPOR2) gene is associated with coronary artery disease and increased ADIPOR2 expression in peripheral monocytes. Cardiovasc Diabetol, 2010; 9: 10
26) Otsuka H, Yanai M, Kobayashi H, Haketa A, Hara M, Sugama K, Kato K, and Soma M: High-molecular-weight adiponectin levels in healthy, community-dwelling, elderly Japanese volunteers: a 5-year prospective observational study. Aging Clin Exp Res, 2018; 30: 791-798
27) Eckardt KU, Berns JS, Rocco MV, and Kasiske BL: Definition and classification of CKD: The debate should be about patient prognosis—a position statement from KDOQI

and KDIGO. Am J Kidney Dis, 2009; 53: 915-920
28) Liu L, Zhong R, Wei S, Yin JY, Xiang H, Zou L, Chen W, Chen JG, Zheng XW, Huang LJ, Zhu BB, Chen Q, Duan SY, Rui R, Yang BF, Sun JW, Xie DS, Xu YH, Miao XP, and Nie SF: Interactions between genetic variants in the adiponectin, adiponectin receptor 1 and environmental factors on the risk of colorectal cancer. PLoS One, 2011; 6: e27301
29) Mather KJ, Christophi CA, Jablonski KA, Knowler WC, Goldberg RB, Kahn SE, Spector T, Dastani Z, Waterworth D, Richards JB, Funahashi T, Pi-Sunyer FX, Pollin TI, Florez JC, and Franks PW: Diabetes Prevention Program Research Group. Common variants in genes encoding adiponectin (ADIPOQ) and its receptors (ADIPOR 1/2), adiponectin concentrations, and diabetes incidence in the Diabetes Prevention Program. Diabet Med, 2012; 29: 1579-1588
30) Siitonen N, Pulkkinen L, Mager U, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Tuomilehto J, Laakso M, and Uusitupa M: Association of sequence variations in the gene encoding adiponectin receptor 1 (ADIPOR1) with body size and insulin levels. The Finnish Diabetes Prevention Study. Diabetologia, 2006; 49: 1795-1805
31) Wang F, Suo S, Sun L, Yang J, Yang F, Zhao C, Li X, Yuan L, Yu S, Qi T, Zhu X, Yuan H, Jin Z, Pu L, Liu D, Sui X, and Yang Z: Analysis of the relationship between ADIPOR1 variants and the susceptibility of chronic metabolic diseases in a Northeast Han Chinese population. Genet Test Mol Biomarkers, 2016; 20: 81-85
32) Kim HY, Hwang JY, Han BG, Lee JY, Park EK, Kim BJ, Lee SH, Kim GS, Kim SY, and Koh JM: Association of ADIPOR1 polymorphisms with bone mineral density in postmenopausal Korean women. Exp Mol Med, 2012; 44: 394-402
33) Han Q, Shu Z, Liang X, Mi R, Yang L, and Li P: Relationship between adiponectin receptor 1 gene polymorphisms and ischemic stroke. Int J Clin Exp Med, 2015; 8: 16719-16723
34) Siitonen N, Pulkkinen L, Lindström J, Kolehmainen M, Schwab U, Eriksson JG, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Tuomilehto J, Uusitupa M. Association of ADIPOR2 gene variants with cardiovascular disease and type 2 diabetes risk in individuals with impaired glucose tolerance: the Finnish Diabetes Prevention Study. Cardiovasc Diabetol, 2011; 10: 83
35) Castilhos JK, Sprinz E, Lazzaretti RK, Kuhmmer R, and Mattevi VS: Polymorphisms in adiponectin receptor genes are associated with lipodystrophy-related phenotypes in HIV-infected patients receiving antiretroviral therapy. HIV Med, 2015; 16: 494-501
36) Nakayama T, Soma M, Rahmutula D, Ozawa Y, and Kanmatsuse K: Isolation of the 5'-flanking region of genes by thermal asymmetric interlaced polymerase chain reaction. Med Sci Monit, 2001; 7: 345-349
37) Sano M, Kuroi N, Nakayama T, Sato N, Izumi Y, Soma M, Kokubun S: Association study of calcitonin-receptor-like receptor gene in essential hypertension. Am J Hypertens, 2005; 18: 403-408
38) Livak KJ, Marmaro J, and Todd JA: Towards fully automated genome-wide polymorphism screening. Nat Genet, 1995; 9: 341-342
39) Mitsnefes M, Kartal J, Khoury P, aand Daniels S: Adiponectin in children with chronic kidney disease: role of adiposity and kidney dysfunction. Clin J Am Soc Nephrol, 2007; 2: 46-50
40) Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, Malatino LS, Bonanno G, Seminara G, Rapisarda F, Fatuzzo P, Buemi M, Nicocia G, Tanaka S, Ouchi N, Kihara S, Funahashi T, and Matsuzawa Y: Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol, 2002; 13: 134-141
41) Huang JW, Yen CJ, Chiang HW, Hung KY, Tsai TJ, and Wu KD: Adiponectin in peritoneal dialysis patients: a comparison with hemodialysis patients and subjects with normal renal function. Am J Kidney Dis, 2004; 43: 1047-1055
42) Kollerits B, Fliser D, Heid IM, Ritz E, and Kronenberg F: MMKD Study Group. Gender-specific association of adiponectin as a predictor of progression of chronic kidney disease: the Mild to Moderate Kidney Disease Study. Kidney Int, 2007; 71: 1279-1286
43) Ohashi K, Iwatani H, Kihara S, Nakagawa Y, Komura N, Fujita K, Maeda N, Nishida M, Katsube F, Shimomura I, Ito T, and Funahashi T: Exacerbation of albuminuria and renal fibrosis in subtotal renal ablation model of adiponectin-knockout mice. Arterioscler Thromb Vasc Biol, 2007; 27: 1910-1917
44) Sharma K, Ramachandrarao S, Qiu G, Usui HK, Zhu Y, Dunn SR, Ouedraogo R, Hough K, McCue P, Chan L, Falkner B, and Goldstein BJ: Adiponectin regulates albuminuria and podocyte function in mice. J Clin Invest, 2008; 118: 1645-1656. DOI: 10.1172/JCI32691
45) Kacso I, Lenghel A, Bondor CI, Moldovan D, Rusu C, Nita C, Hancu N, Gherman Caprioara M, and Kacso G: Low plasma adiponectin levels predict increased urinary albumin/creatinine ratio in type 2 diabetes patients. Int Urol Nephrol, 2012; 44: 1151-1157
46) Zha D, Wu X, Gao P. Adiponectin and its receptors in diabetic kidney disease: molecular mechanisms and clinical potential. Endocrinology, 2017; 158: 2022-2034
47) Richardson DK, Schneider J, Fourcaudot MJ, Rodriguez LM, Arya R, Dyer TD, Almasy L, Blangero J, Stern MP, Defronzo RA, Duggirala R, and Jenkinson CP: Association between variants in the genes for adiponectin and its receptors with insulin resistance syndrome (IRS)-related phenotypes in Mexican Americans. Diabetologia, 2006; 49: 2317-2328
48) Kotronen A, Yki-Jarvinen H, Aminoff A, Bergholm R, Pietilainen KH, Westerbacka J, Talmud PJ, Humphries SE, Hamsten A, Isomaa B, Groop L, Orho-Melander M, Ehrenborg E, and Fisher RM: Genetic variation in the ADIPOR2 gene is associated with liver fat content and its surrogate markers in three independent cohorts. Eur J Endocrinol, 2009; 160: 593-602
49) Eddy AA: Interstitial macrophages as mediators of renal fibrosis. Exp Nephrol, 1995; 3: 76-79
50) Nishida M, Okumura Y, Fujimoto S, Shiraishi I, Itoi T, and Hamaoka K. Adoptive transfer of macrophages ameliorates renal fibrosis in mice. Biochem Biophys Res Commun, 2005; 332: 11-16

51) Klessens CQF, Zandbergen M, Wolterbeek R, Bruijn JA, Rabelink TJ, Bajema IM, and IJpelaar DHT: Macrophages in diabetic nephropathy in patients with type 2 diabetes. Nephrol Dial Transplant, 2017; 32: 1322-1329
52) Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med, 2007; 13: 332-339
53) Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, Yamaguchi M, Tanabe H, Kimura-Someya T, Shir- ouzu M, Ogata H, Tokuyama K, Ueki K, Nagano T, Tanaka A, Yokoyama S, Kadowaki T. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature, 2013; 503: 493-499
54) Bluher M, Bullen JW Jr, Lee JH, Kralisch S, Fasshauer M, Kloting N, Niebauer J, Schon MR, Williams CJ, and Mantzoros CS: Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: associations with metabolic parameters and insulin resistance and regulation by physical training. J Clin Endocrinol Metab, 2006; 91: 2310-2316
55) Nannipieri M, Cecchetti F, Anselmino M, Mancini E, Marchetti G, Bonotti A, Baldi S, Solito B, Giannetti M, Pinchera A, Santini F, and Ferrannini E: Pattern of expression of adiponectin receptors in human liver and its relation to nonalcoholic steatohepatitis. Obes Surg, 2009; 19: 467-474
56) Felder TK, Hahne P, Soyal SM, Miller K, Hoffinger H, Oberkofler H, Krempler F, and Patsch W: Hepatic adiponectin receptors (ADIPOR) 1 and 2 mRNA and their relation to insulin resistance in obese humans. Int J Obes, 2010; 34: 846-851
57) Kollias A, Tsiotra PC, Ikonomidis I, Maratou E, Mitrou P, Kyriazi E, Boutati E, Lekakis J, Economopoulos T, Kremastinos DT, Dimitriadis G, and Raptis SA: Adiponectin levels and expression of adiponectin receptors in isolated monocytes from overweight patients with coronary artery disease. Cardiovasc Diabetol, 2011; 10: 14

Supplementary Table 1. MAF and HWE *p*-values for the studied ADIPOR1 and ADIPOR2 SNPs

SNP ID (rs#)	CHR	BP	CR	MAF	minor	major	HWE
rs7539542	1	202940846	1.000	0.2018	C	G	0.8512
rs1342387	1	202945228	1.000	0.4564	C	T	1.0000
rs16850797	1	202947555	1.000	0.3218	C	G	1.0000
rs12045862	1	202947678	1.000	0.3691	C	T	0.6046
rs16850799	1	202950723	1.000	0.4982	A	G	0.1490
rs2275737	1	202951172	1.000	0.2382	A	C	1.0000
rs1539355	1	202954952	1.000	0.1255	G	A	0.4036
rs11061925	12	1694068	1.000	0.3327	T	C	0.1358
rs10773983	12	1722080	1.000	0.3527	A	G	0.2340
rs10773986	12	1732158	1.000	0.4927	G	A	0.9043
rs10773988	12	1747015	1.000	0.44	G	A	0.7135
rs12230440	12	1747787	1.000	0.2745	G	T	0.5442

MAF, minor allele frequency; HWE, Hardy Weinberg Equilibrium; ADIPOR, adiponectin receptor; SNP, single nucleotide polymorphism; CHR, chromosome; CR, call rate.

Supplementary Table 2. Analysis of recessive and dominant models for rs12230440

	rs12230440			*P* value for GG + GT vs TT	*P* value for GG + GT vs TT (Bonferroni Corr.)	*P* value for GG vs GT + TT	*P* value for GG vs GT + TT (Bonferroni Corr.)
	GG	GT	TT				
Age, years	72.9 (71.4-76.0)	74.0 (69.0-77.0)	73.0 (68.0-77.0)	0.98	1	0.98	1
Sex, male %	30.4% (7/23)	27.5% (28/102)	28.8% (42/146)	0.89	1	0.82	1
Body mass index, kg/m^2	22.9 (21.0-24.8)	23.2 (21.1-24.9)	22.9 (21.4-24.6)	0.82	1	0.84	1
Current smoking status, %	17.4% (4/23)	9.8% (10/102)	13.7% (20/146)	0.54	1	0.46	1
SBP, mmHg (n=268)	146.0 (132.0-154.0)	143.0 (130.0-155.3)	143.0 (131.3-154.0)	0.86	1	0.81	1
DBP, mmHg (n=270)	79.0 (74.0-86.0)	76.0 (69.0-85.0)	77.0 (70.5-82.0)	0.28	1	0.065	0.78
MAP, mmHg (n=269)	100.3 (95.0-107.7)	97.5 (90.9-108.0)	98.2 (91.4-108.0)	0.56	1	0.30	1
GA, %	14.3 (13.4-15.4)	15.0 (14.1-16.1)	15.2 (14.5-16.1)	0.094	1	0.014	0.17
Total cholesterol, mg/dL	214.0 (180.0-237.0)	200.0 (177.5-227.3)	204.0 (181.0-224.0)	0.98	1	0.42	1
HDL cholesterol, mg/dL	54.5 (40.8-71.0)	53.0 (465.0-64.3)	56.0 (46.0-63.8)	0.75	1	0.83	1
LDL cholesterol, mg/dL	118.8 (100.4-143.6)	115.0 (95.4-140.3)	118.0 (99.9-138.0)	0.98	1	0.67	1
TG, mg/dL	152.5 (105.8.0-254.5)	128.5 (96.0-177.5)	132.5 (92.0-173.8)	0.56	1	0.075	0.90
Total adiponectin, ng/mL	9.8 (8.2-18.9)	12.1 (8.7-17.6)	11.8 (8.8-17.9)	0.85	1	0.69	1
HMW adiponectin, ng/mL	6.2 (3.3-10.9)	7.2 (4.5-10.5)	7.0 (4.4-10.8)	0.68	1	0.49	1
Cystatin C, mg/L	1.01 (0.98-1.16)	0.91 (0.83-1.11)	0.93 (0.82-1.02)	0.060	0.72	0.0017	0.020
eGFRcys, mL/min/1.73 m^2	64.4 (55.2-69.0)	72.8 (60.0-83.0)	70.9 (63.8-82.9)	0.080	0.96	0.0021	0.025
Antihypertensive medication, %	56.5 (13/23)	34.3 (35/102)	7.5 (11/146)	0.65	1	0.088	1
Antidiabetic medication, %	0 (0/23)	8.8 (9/102)	9.1 (5/55)	0.92	1	0.16	1

Data are shown as medians (25th and 75th percentile). Bonferroni Corr, Bonferroni Correction; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; GA, glycated albumin; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; TG, triglyceride; HMW adiponectin, high molecular weight adiponectin; eGFRcys, cystatin C-based estimated glomerular filtration rate.