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Sex-specific impact of *GCKR* rs1260326 polymorphism on metabolic traits in an older Japanese population: the Bunkyo Health Study

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Abstract

Background: Metabolic syndrome involves health problems influenced by aging and genetics. The glucokinase regulatory protein (*GCKR*) rs1260326 polymorphism (Leu446) is associated with metabolic traits. This study explores the impact of the GCKR rs1260326 polymorphism on metabolic traits in older Japanese with focusing on sex-specific differences.

Methods: This cross-sectional study from the Bunkyo Health Study in Tokyo, Japan, examined 883 participants aged 65–84 years. Participants were excluded with diabetes, or on drug treatment for diabetes or dyslipidemia. The *GCKR* P446L polymorphism was analyzed and compared their characteristics of physical activity, dietary intake, body composition, and metabolic parameters.

Results: Study participants with *GCKR* rs1260326 genotypes (C/C 20.7%, C/T 47.6%, T/T 31.7%) had a median age of 72 years, and 60.4% were women. Men with the T/T genotype, as compared to the C/C genotype, had a lower body weight, body mass index (BMI), and skeletal mass index. This genotype also associated with lower fasting insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), and higher Matsuda index, but not after adjustment for age, BMI, and physical activity. In contrast, women with the T/T genotype, compared to the C/C genotype, showed higher C-reactive protein, fibroblast growth factor 21, and Matsuda index. They also had lower fasting insulin, insulin area under the curve, and HOMA-IR; with these associations being independent of age, BMI, and physical activity.

Conclusion: The *GCKR* rs1260326 genotype-affected metabolic traits differentially by sex in older Japanese. This highlights the need to consider sex differences in *GCKR*-related metabolic outcomes.

Plain language summary

How a certain gene change affects health differently for older men and women in Japan: a study from Bunkyo

This study looks into how a certain genetic change affects the health of older Japanese people, focusing on whether there are differences between men and women. It involves participants aged 65 to 84 from Tokyo who are not taking medication for diabetes or dyslipidemia. The findings indicate that this genetic variation impacts men and women differently. Men with a specific version of this genetic change tend to have lower body weight, body mass index (BMI), and less skeletal mass. They also show lower insulin

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levels and resistance, but these associations weaken when considering age, BMI, and physical activity. On the other hand, women with this genetic variation showed higher levels of markers indicating inflammation and metabolic health, alongside better insulin sensitivity. These relationships held even after adjusting for age, BMI, and activity levels. From this research, it's clear that the effects of this genetic change on health vary between older Japanese men and women. This suggests the importance of considering gender differences when studying how genes influence our health, underlining the need for personalized approaches in understanding and managing health issues.

Keywords: *GCKR* rs1260326, insulin sensibility, sex differences

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Introduction

Metabolic syndrome is a condition characterized by multiple metabolic disorders, including visceral obesity, hypertension, hyperglycemia, insulin resistance, and dyslipidemia.[1] Aging significantly increases the prevalence of these metabolic disorders,[2,3] and their presence in older adults is associated with cardiovascular disease,[4] cognitive decline,[5] frailty,[6] and higher mortality.[4] For example, the risk of high blood glucose levels is about 38 times higher in older adults aged 70–75 years compared to those aged 40–45 years.[2] The characteristics of metabolic diseases are influenced not only by environmental factors, such as excessive calorie intake and sedentary lifestyle, but also by interactions with genetic factors.[7] Since lifestyle habits often change with age,[8,9] the impact of genetic factors on metabolic diseases may differ in older adults compared with younger ones. However, the specific effects of certain genetic polymorphisms on metabolic disease remain poorly understood in older adults.

The prevalence of metabolic disease in Japan shows a large sex-specific difference.[3] For example, a higher prevalence of metabolic syndrome was observed in men compared with women (men 45.7%, women 15.8%).[10] The prevalence of diabetes in Japan was also much higher in men (9.63%) than in women (5.33%).[11] Thus, the significant difference in the prevalence of metabolic disease between men and women in Japan highlights the importance of exploring how genetic factors influence these conditions in each sex.

The T allele (Leu446) of the rs1260326 polymorphism (T/C), a functional missense variant in the glucokinase regulatory protein gene (*GCKR*), affects glucose uptake and release in the liver. Studies in populations of European descent, including those without diabetes, have shown that this allele is associated with reduced fasting glucose,[12–14] fasting insulin,[12,14], insulin resistance,[14] blood triglycerides (TG),[15] C-reactive protein (CRP),[16,17] and type 2 diabetes.[14,18] On the other hand, the metabolic effects of the *GCKR* rs1260326 polymorphism in the older Japanese population are still poorly understood, although lower fasting glucose in older individuals has been reported in East Asians.[19,20] Hence, it is particularly important to investigate the metabolic effects of the *GCKR* rs1260326 polymorphism in the older Japanese population, including examining sex-related differences.

Against this background, our study explored how the T allele (Leu446) of the rs1260326 polymorphism in the *GCKR* gene affected older Japanese adults, considering both men and women. Previous studies have mainly focused on general populations in Europe, and this study investigated whether identical genetic changes also affected metabolism in older Japanese. It is also important to investigate sex differences, as the prevalence of metabolic diseases differs significantly between Japanese men and women. A better understanding of these genetic influences could lead to more effective management and treatment of metabolic disease in the older Japanese population.

Research design and methods

Study design and participants

This cross-sectional study used baseline data from the Bunkyo Health Study.[21] Briefly, in this study, we recruited individuals aged between 65 and

84 years living in Bunkyo-ku, an urban area in Tokyo, Japan. The Bunkyo Health Study registry initially enrolled 1629 individuals. To minimize the confounding effects of disease and medication, we excluded 710 participants from this cohort who were diagnosed with diabetes mellitus or were taking oral medication for diabetes or dyslipidemia.[22] Additionally, 20 of the remaining 919 participants were excluded due to the unavailability of specific data (body composition ($n=12$), glycemic control indicators ($n=5$), genotype ($n=3$)), and a further 16 were excluded due to outlier levels of serum fibroblast growth factor 21 (FGF21). These outliers of FGF21 were defined as values falling outside the mean ± 3 standard deviations (3σ principle), following the approach used in previous studies,[23,24] to prevent skewing the statistical analysis. Consequently, the final sample for this analysis comprised 883 participants (350 men and 533 women; Figure S1). All participants completed study examinations at the Sportology Center of Juntendo University from October 15, 2015 to October 1, 2018. The study protocol was approved by the ethics committee of Juntendo University in November 2015 (Nos. 2015078, 2016138, 2016038, 2017121, and 2019085: Supplemental Appendix A). Subjects underwent measurements over 2 days. On the first day, physical activity and dietary intake were assessed by questionnaire; on the second day, after an overnight fast, abdominal fat distribution was assessed by MRI and glucose tolerance was evaluated using a 75-g oral glucose tolerance test (OGTT). This study was carried out in accordance with the principles outlined in the Declaration of Helsinki. All participants gave written informed consent and were informed that they had the right to withdraw from the trial at any time.

Genotyping

Genomic DNA was isolated from whole blood using the standard method (RNeasy Blood and Tissue DNA Extraction Kit; QIAGEN, Minneapolis, MN, USA). All participants were genotyped for the *GCKR* P446L (rs1260326) polymorphism using the Infinium Asian Screening Array-24 v1.0 BeadChip (Illumina, San Diego, CA, USA).

Other measurements

Physical activity (PA) level was evaluated using the International Physical Activity Questionnaire (IPAQ), which assesses different types of physical activity, such as walking and both moderate- and high-intensity activities.[25] The detailed IPAQ can be found in Supplemental Appendix B. As part of the survey on dietary history, participants completed the Brief Diet History Questionnaire (BDHQ).[26,27] The IPAQ has been validated to assess physical activity levels in various populations, including the older people and certain cohorts[28–30] and BDHQ has been validated to assess dietary intake in various populations including the elderly in Japanese.[26,31,32] The skeletal muscle mass index (SMI; appendicular skeletal muscle mass/height^2) and percent body fat (PBF) were evaluated by bioelectrical impedance analysis (InBody770, InBody Inc., Tokyo, Japan). Visceral fat area (VFA) and subcutaneous fat area (SFA) were measured with a 0.3-T MR scanner (AIRIS Vento; Hitachi, Tokyo, Japan). Using the intervertebral space between the fourth and fifth lumbar vertebrae (L4-L5) as the point of origin, transverse images of 10-mm slice thickness were obtained every 100 mm from head to foot, resulting in a total of 10 images for each subject. All MRI data were transferred to a computer workstation for analysis using specialized image analysis software (AZE Virtual Place; AZE, Tokyo, Japan) to analyze VFA and SFA. Biochemical test indices were tested at a contracted clinical laboratory (SRL Corporation, Tokyo, Japan).

Evaluation of glucose metabolism

The cut-off values for impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were those specified by the World Health Organization (WHO, 1999) criteria[33]: IFG, fasting plasma glucose (FPG) 110–126 mg/dL and 2-h postload glucose <200 mg/dL; and IGT, FPG <126 mg/dL and 2-h postload glucose 140–200 mg/dL. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as fasting insulin (μU/mL) × fasting glucose (mg/dL)/405.[34] The Matsuda index was calculated as 10,000/square root of (fasting glucose (mg/dL) × fasting insulin (μU/mL)) × (mean glucose (mg/dL) × mean insulin during OGTT (μU/mL)).[35] The homeostasis model assessment of beta cell function (HOMA-β) was calculated as fasting insulin (μU/mL) × 360/(fasting glucose (mg/dL) − 63).[34] The area under the curve (AUC) of glucose and insulin profile during OGTT were calculated.

Statistical analysis

Statistical analysis was performed using SPSS statistical software ver. 28.0 (IBM SPSS Statistics,

IBM Corp., Armonk, NY, USA). Subject background data were expressed as median (interquartile range (IQR)) or frequency (%), and sex differences were tested by the Mann–Whitney *U* test and χ^2 test. All data below are presented as means ± SD. Analyses of associations between *GCKR* polymorphisms and clinical data stratified by sex were performed by one-way analysis of variance (ANOVA), and those of associations between *GCKR* polymorphisms and blood parameters were performed by analysis of covariance (ANCOVA). The Bonferroni method was used for posttests. Because body mass index (BMI),[36] physical activity,[37] and age[38] have been shown to increase the likelihood of developing insulin resistance, covariates were adjusted for these factors. All statistical tests were two-tailed at the 5% significance level.

Results

Participant characteristics

Tables 1 and 2 show participant characteristics. The overall median age of the study population was 72 years (IQR: 68, 76 years), and 60.4% of participants were women (Table 1). The overall genotype frequencies of the rs1260326 polymorphism were C/C 20.7%, C/T 47.6%, and T/T 31.7% (Table 1). Similar to previous studies in East Asian populations, the present subjects demonstrated higher T allele frequencies compared with a European population (42.4%).[39] Genotype frequencies in this study were similar between men and women. Men had greater height, weight, BMI, SMI, VFA, energy intake, and alcohol intake compared with women ($p < 0.05$; Table 1). In addition, levels of TG, fasting glucose, CRP, and FGF21 were higher in men than in women ($p < 0.05$), whereas PBF, SFA, HOMA-β, and levels of adiponectin and fasting free fatty acids were lower in men ($p < 0.05$; Tables 1 and 2). No sex differences were found in insulin level, HOMA-IR, Matsuda index values, or the prevalence of glucose metabolism disorders (Table 2). Approximately 70% of both sexes exhibited normal glucose tolerance (NGT; Table 2).

Clinical characteristics of the study participants by GCKR genotype in men and women

Table 3 shows the clinical characteristics of participants with each *GCKR* genotype after stratification by sex. In men, the *GCKR* rs1260326 T/T genotype, compared with the C/C genotype, had lower body weight (62.0 ± 7.6 kg vs 66.0 ± 7.1 kg, respectively, $p = 0.001$), BMI (22.6 ± 2.2 kg/m^2 vs 23.8 ± 2.3 kg/m^2, respectively, $p = 0.001$), and SMI (7.2 ± 0.6 kg/m^2 vs 7.5 ± 0.6 kg/m^2, respectively, $p = 0.013$), while PBF showed significant differences between the *GCKR* rs1260326 genotypes ($p < 0.05$). In women, PA showed significant differences between the *GCKR* rs1260326 genotypes ($p < 0.05$). There were no significant differences in dietary intake between the *GCKR* rs1260326 genotypes in either sex.

Associations between GCKR genotype and metabolic parameters in men and women

The associations between *GCKR* rs1260326 genotype and metabolic parameters are shown in Tables 4 and 5. In men, the T/T *GCKR* rs1260326 genotype, compared with the C/C genotype, was associated with lower fasting insulin (4.1 ± 0.3 μU/mL vs 5.1 ± 0.3 μU/mL, respectively, $p = 0.039$) and HOMA-IR (1.0 ± 0.07 vs 1.3 ± 0.08, respectively, $p = 0.028$), and higher Matsuda index (8.7 ± 0.4 vs 7.2 ± 0.5, respectively, $p = 0.038$), but these associations were not present in ANCOVA adjusted for age, BMI, and PA. In women, on the other hand, the T/T genotype compared with the C/C genotype was associated in ANOVA with higher levels of CRP (969.0 ± 137.6 mg/dL vs 457.3 ± 173.9 mg/dL, respectively, $p < 0.001$), FGF21 (285.4 ± 11.4 pg/mL vs 240.6 ± 14.4 pg/mL, respectively, $p = 0.033$), and Matsuda index (8.8 ± 0.3 vs 7.7 ± 0.4, respectively, $p = 0.033$), and lower levels of fasting insulin (3.8 ± 0.2 μU/mL vs 4.7 ± 0.3 μU/mL, respectively, $p = 0.024$), insulin AUC (4,993.3 ± 239.3 vs 5967.9 ± 302.5, respectively, $p = 0.015$), and HOMA-IR (0.9 ± 0.05 vs 1.1 ± 0.07, respectively, $p = 0.020$). These associations in women were also present in ANCOVA adjusted for age, BMI, and PA. There were no associations between *GCKR* rs1260326 genotype and the level of TG or adiponectin in either sex.

Discussion

This study aimed to determine the associations between the *GCKR* rs1260326 polymorphism and both metabolic-related parameters and sex differences in an older Japanese population. The *GCKR* rs1260326 genotype frequencies of the study participants (C/C 20.7%, C/T 47.6%, and T/T 31.7%: alternative allele frequency 55.5%) were similar to those previously reported in an

Table 1. Clinical characteristics of the study participants stratified by sex.

Participant characteristics	Men	Women	All
n, %	350 (39.6)	533 (60.4)	883
Age, years	72 (68, 77)	72 (68, 76)	72 (68, 76)
Weight, kg	63.7 (57.7, 68.9)*	49.7 (45.2, 54.5)	54.3 (48.2, 62.7)
Height, cm	166.2 (161.6, 169.7)*	152.5 (149.1, 156.1)	159.9 (151.6, 165.4)
BMI, kg/m^2	22.9 (21.2, 24.8)*	21.5 (19.5, 23.4)	22.1 (20.1, 24.1)
PBF, %	23.4 (19.6, 27)*	30 (24.1, 34.1)	26.7 (21.9, 32.3)
SMI, kg/m^2	7.3 (6.9, 7.8)*	5.7 (5.4, 6.1)	6.2 (5.6, 7.2)
SFA, cm^3	116.5 (89.2, 148.0)*	147.8 (115.3, 197.9)	134.1 (102.9, 179.7)
VFA, cm^3	77.0 (57.5, 100.3)*	56.8 (38.3, 80.2)	65.9 (43.7, 89.6)
GCKRrs1260326			
C/C, *n* (%)	76 (21.7)	107 (20.1)	183 (20.7)
C/T, *n* (%)	165 (47.1)	255 (47.8)	420 (47.6)
T/T, *n* (%)	109 (31.1)	171 (32.1)	280 (31.7)
PA, Met-hours/week	35.1 (19.9, 63.8)	29.7 (16.5, 53.2)	32.3 (17.3, 57.8)
Energy intake, kcal	2085.8 (1687.9, 2531.5)*	1776.4 (1452.3, 2158.9)	1881.9 (1535.9, 2301.1)
Protein intake (%energy), %	15.4 (13.5, 17.5)*	17.5 (15.4, 19.7)	16.5 (14.6, 19)
Fat intake (%energy), %	26.8 (23, 30)*	29.6 (25.3, 33.4)	28.2 (24.3, 32.2)
Carbohydrate intake (%energy), %	48.9 (43.3, 54.7)*	50.1 (44.8, 55.6)	49.8 (44.1, 55.2)
Alcohol intake, g/day	11.6 (0.2, 36.9)*	0.1 (0, 3.1)	0.8 (0, 15.2)

Data are expressed as median (interquartile range) for continuous variables and frequencies (percentage) for categorical variables.
*<0.05 versus women by Mann–Whitney *U* test and χ^2 test.
BMI, body mass index; GCKR, glucokinase regulatory protein; PA, physical activity; PBF, percent body fat; SFA, subcutaneous fat area; SMI, skeletal muscle mass index; VFA, visceral fat area.

Asian population.[39] Differences in traits between participants with different *GCKR* rs1260326 genotypes were sex dependent, affecting body composition in men and PA in women. Men with the T/T genotype showed higher insulin sensitivity than those with the C/C genotype, but this difference was not significant after adjusting for confounding factors. In contrast, women with the T/T genotype had higher CRP and FGF21 levels, as well as greater insulin sensitivity than those with the C/C genotype, and these differences remained significant even after adjustments. In both men and women, neither genotype was associated with dietary intake or the level of TG or adiponectin.

In this study, *GCKR* genotype was associated with body composition in men and PA in women. An association between the *GCKR* rs780094 minor allele in linkage disequilibrium with rs1260326 and low BMI or high PA has been reported.[16,19,40] These studies showed no sex differences but still support the present findings.

Table 2. Blood test data of the study participants stratified by sex.

Biochemical parameters	Men	Women	All
TG, mg/dL	84.0 (63.0, 119.0)*	76.0 (58.0, 106.0)	79.0 (60.0, 110.0)
HsCRP, mg/dL	386.0 (200.0, 866.0)*	353.0 (178.5, 687.0)	363.0 (186.0, 738.0)
Adiponectin, μg/mL	9.3 (6.7, 12.9)*	13.7 (10.5, 19.9)	12.1 (8.5, 16.7)
FGF21, pg/mL	268.4 (175.4, 399.7)*	228.7 (161.1, 339.9)	242.2 (164.3, 356.2)
AST, U/L	22.0 (18.8, 26.0)	21.0 (19.0, 25.0)	22.0 (19.0, 25.0)
ALT, U/L	17.0 (13.0, 21.0)	15.0 (13.0, 19.0)	16.0 (13.0, 20.0)
γ-GTP, IU/L	26.0 (18.0, 38.0)	18.0 (14.0, 24.0)	20.0 (15.0, 31.0)
Fasting insulin, μU/mL	3.7 (2.6, 5.5)	3.6 (2.5, 5.1)	3.6 (2.6, 5.2)
IRI AUC	4415.1 (3162.8, 6758.4)	4697.8 (3611.6, 6386.4)	4605.9 (3456.5, 6536.1)
Fasting glucose, mg/dL	96.0 (91.0, 102.0)*	93.0 (88.0, 98.0)	94.0 (89.0, 100.0)
Glucose AUC	17557.5 (15337.5, 19740)	16537.5 (14418.8, 18930)	16995 (14756.3, 19297.5)
Fasting FFAs, μEq/L	445.0 (357.9, 576.4)*	490.3 (376.5, 631.2)	468.0 (369.0, 602.0)
HOMA-IR	0.9 (0.6, 1.4)	0.8 (0.6, 1.2)	0.9 (0.6, 1.2)
HOMA-β, %	40.7 (28.9, 57.1)*	45.0 (32.1, 59)	43.6 (30.9, 58.6)
Matsuda index	7.3 (4.8, 10.2)	7.5 (5.4, 10.2)	7.5 (5.2, 10.2)
WHO			
NGT, *n* (%)	238 (68.2)	365 (68.6)	603 (68.4)
IFG, *n* (%)	7 (2.0)	6 (1.1)	13 (1.5)
IGT, *n* (%)	104 (29.8)	161 (30.3)	265 (30.1)

Data are expressed as median (interquartile range) for continuous variables and frequencies (percentage) for categorical variables.
*<0.05 versus women by Mann–Whitney *U* test and χ^2 test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; FFA, free fatty acid; FGF21, fibroblast growth factor 21; GCKR, glucokinase regulatory protein; HOMA-IR, homeostasis model assessment of insulin resistance index; HOMA-β, homeostasis model assessment of beta cell function; HsCRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; NGT, normal glucose tolerance; TG, triglycerides; γ-GTP, gamma glutamyl transferase.

Interestingly, this study identified a sex difference in the association between the *GCKR* T allele and metabolic parameters, which had been strongly suggested in prior studies.[12–17] Specifically, although there were no significant differences in HOMA-β between genotypes, the GCKR rs1260326 T/T genotype was associated with lower HOMA-IR and higher Matsuda index values in either sex, the GCKR rs1260326 polymorphism had a limited effect on β-cell function, although it was highly associated with insulin sensitivity. Furthermore, this study showed that in men there was a BMI-dependent association between *GCKR* rs1260326 genotype and metabolic parameters, whereas in women, the association was independent. These sex differences might be related to a discrepancy in adipose distribution, with men tending to have more visceral adipose tissue than women at a given BMI.[41,42] Although the exact mechanism is unclear, these findings highlight the influence of sex on the effects of the *GCKR* T allele and emphasize the importance of sex-specific considerations in understanding metabolic outcomes.

Table 3. Clinical characteristics of the study participants stratified by *GCKR* genotype and sex.

GCKRrs1260326 (*n*)	Men				Women			
	C/C (76)	C/T (165)	T/T (109)	*p* Value	C/C (107)	C/T (255)	T/T (171)	*p* Value
Age, years	71.7 ± 5.1	72.8 ± 5.3	72.3 ± 5.4	0.318	73.1 ± 5.4	72.5 ± 5.2	72.0 ± 5.4	0.237
Weight, kg	66.0 ± 7.1	63.6 ± 8.7	62.0 ± 7.6 a	**0.001**	50.5 ± 8.4	50.4 ± 7.3	50.1 ± 7	0.839
Height, cm	166.3 ± 5.2	166.2 ± 5.8	165.6 ± 5.6	0.581	152.6 ± 5.5	152.7 ± 5.9	152.2 ± 5.2	0.677
BMI, m^2	23.8 ± 2.3	23.0 ± 2.7 a	22.6 ± 2.2 a	**0.001**	21.6 ± 3.3	21.6 ± 3.0	21.6 ± 3.0	0.994
PBF, %	24.7 ± 5.2	23.0 ± 6.2	23.0 ± 5.7	**0.048**	29.3 ± 7.3	29.7 ± 6.9	29.6 ± 7.0	0.907
SMI, kg/m^2	7.5 ± 0.6	7.3 ± 0.6	7.2 ± 0.6 a	**0.013**	5.7 ± 0.6	5.8 ± 0.6	5.7 ± 0.5	0.946
SFA, cm^3	134.7 ± 44.5	117.9 ± 47.5 a	116.6 ± 43.7 a	**0.012**	155.6 ± 63.1	155.7 ± 57.9	153.5 ± 59.5	0.924
VFA, cm^3	84.9 ± 35.4	83.5 ± 42.9	77.7 ± 34	0.302	65.3 ± 36.8	61.5 ± 31.5	63.6 ± 31.8	0.595
PA, Met-hours/week	60.5 ± 67.5	46.3 ± 45.6	63.4 ± 88.6	0.073	35.8 ± 35.2	47.5 ± 51.0	43.2 ± 43.0	**0.043**
Energy intake, g/day	2170.4 ± 699.9	2159.2 ± 619.2	2174.3 ± 608.7	0.979	1895.3 ± 627.7	1859.8 ± 536.2	1811.8 ± 506.2	0.450
Protein intake (%energy)	15.9 ± 3.1	15.7 ± 3.2	15.5 ± 3.0	0.748	17.4 ± 3.4	17.8 ± 3.2	17.6 ± 3.3	0.650
Fat intake (%energy)	26.7 ± 5.1	26.7 ± 6	26.4 ± 5.5	0.922	29.0 ± 5.5	29.7 ± 5.3	29.0 ± 5.9	0.331
Carbohydrate intake (%energy)	48.9 ± 8.2	48.5 ± 7.7	49.4 ± 9.1	0.695	50.6 ± 8.2	49.8 ± 7.4	50.0 ± 8.7	0.697
Alcohol intake, g/day	26.3 ± 31.2	25 ± 33.7	20.4 ± 23.0	0.253	29.0 ± 5.5	29.7 ± 5.3	29.0 ± 5.9	0.331

Data are expressed as mean ± SD. *p* value, Bold values indicate statistically significant differences ($p < 0.05$) followed by ANOVA for continuous variables.
$p < 0.05$, a versus C/C for Bonferroni post hoc test.
ANOVA, analysis of variance; BMI, body mass index; GCKR, glucokinase regulatory protein; PA, physical activity; PBF, percent body fat; SFA, subcutaneous fat area; SMI, skeletal muscle mass index; VFA, visceral fat area.

Table 4. Association between *GCKR* and metabolic parameters stratified by sex using ANOVA.

GCKRrs1260326 (*n*)	Men				Women			
	C/C (76)	C/T (165)	T/T (109)	*p* value	C/C (107)	C/T (255)	T/T (171)	*p* value
TG, mg/dL	95.5 ± 5.7	94.9 ± 3.9	99.7 ± 4.8	0.706	86.7 ± 4.4	85.1 ± 2.9	91.3 ± 3.5	0.428
HsCRP, mg/dL	866 ± 446.5	1224 ± 303.1	1337.2 ± 372.9	0.359	457.3 ± 173.9	825.0 ± 112.7	969.0 ± 137.6	**<0.001**
Adiponectin, µg/mL	9.6 ± 0.6	10.5 ± 0.4	10.1 ± 0.5	0.390	15.2 ± 0.7	15.8 ± 0.4	15.0 ± 0.5	0.503
FGF21, pg/mL	286.7 ± 21.7	309.7 ± 14.7	334.4 ± 18.1	0.206	240.6 ± 14.4	253.2 ± 9.3	285.4 ± 11.4 a	**0.033**
AST, U/L	23.6 ± 0.9	22.6 ± 0.6	23.0 ± 0.7	0.555	22.6 ± 0.8	22.8 ± 0.5	22.6 ± 0.6	0.952
ALT, U/L	19.9 ± 0.9	18.0 ± 0.6	18.6 ± 0.8	0.219	16.5 ± 0.9	17.8 ± 0.6	16.5 ± 0.7	0.273
γ-GTP, IU/L	35.0 ± 4.6	35.8 ± 3.1	40.7 ± 3.8	0.639	20.1 ± 1.6	23.4 ± 1.1	22.9 ± 1.3	0.115
Fasting insulin, µU/mL	5.1 ± 0.3	4.3 ± 0.2	4.1 ± 0.3 a	**0.039**	4.7 ± 0.3	4.4 ± 0.2	3.8 ± 0.2 a	**0.024**
IRI AUC	5684.9 ± 342.3	5305.3 ± 232.3	5113.7 ± 285.8	0.469	5967.9 ± 302.5	5642.1 ± 196.3	4993.3 ± 239.3 a	**0.015**
Fasting glucose, mg/dL	97.9 ± 0.9	97.4 ± 0.6	96.2 ± 0.8	0.240	93.2 ± 0.7	93.8 ± 0.5	92.4 ± 0.6	0.171
Glucose AUC	17780.1 ± 365.3	17883.7 ± 247.9	17210.6 ± 305.0	0.218	17120.3 ± 322.4	16821.7 ± 209.3	16674.9 ± 255.1	0.569
Fasting FFAs, µEq/L	460.1 ± 19.8	468.2 ± 13.4	484.8 ± 16.5	0.613	510.1 ± 20.2	530.9 ± 13.1	512.7 ± 16.0	0.570
HOMA-IR	1.3 ± 0.08	1.1 ± 0.06	1.0 ± 0.07 a	**0.028**	1.1 ± 0.07	1.0 ± 0.04	0.9 ± 0.05 a	**0.020**
HOMA-β, %	52.7 ± 3	45.4 ± 2	44.6 ± 2.5	0.088	55.5 ± 3.1	52.0 ± 2	47.7 ± 2.4	0.107
Matsuda index	7.2 ± 0.5	8.0 ± 0.3	8.7 ± 0.4 a	**0.038**	7.7 ± 0.4	7.9 ± 0.2	8.8 ± 0.3	**0.033**

Data are expressed as mean ± SD. *p* value, Bold values indicate statistically significant differences ($p < 0.05$) followed by ANCOVA for continuous variables.
$p < 0.05$, a versus C/C for Bonferroni post hoc test.
ALT, alanine aminotransferase; ANOVA, analysis of variance; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; FFA, free fatty acid; FGF21, fibroblast growth factor 21; GCKR, glucokinase regulatory protein; HOMA-IR, homeostasis model assessment of insulin resistance index; HOMA-β, homeostasis model assessment of beta cell function; HsCRP, high-sensitivity C-reactive protein; IRI, immunoreactive insulin; TG, triglycerides; γ-GTP, gamma glutamyl transferase.

Table 5. Association between *GCKR* and metabolic parameters stratified by sex using ANCOVA.

GCKR rs1260326 (*n*)	Men				Women			
	C/C (76)	C/T (165)	T/T (109)	*p* Value	C/C (107)	C/T (255)	T/T (171)	*p* Value
TG, mg/dL	92.0 ± 5.6	94.9 ± 3.8	102.2 ± 4.7	0.322	86.4 ± 4.3	85.3 ± 2.8	91.2 ± 3.4	0.388
HsCRP, mg/dL	724.1 ± 449.3	1207.6 ± 302.5	1461.0 ± 373.4	0.453	436.4 ± 172.8	823.2 ± 111.6	984.8 ± 136.2a	**0.044**
Adiponectin, μg/mL	10.2 ± 0.5	10.5 ± 0.4	9.9 ± 0.5	0.588	15.0 ± 0.6	15.8 ± 0.4	15.1 ± 0.5	0.446
FGF21, pg/mL	280.7 ± 21.8	308.1 ± 14.7	341.0 ± 18.2	0.101	239.9 ± 14.1	252.5 ± 9.1	286.8 ± 11.1 a	**0.015**
AST, U/L	23.3 ± 0.9	22.7 ± 0.6	23.2 ± 0.7	0.770	22.6 ± 0.8	22.8 ± 0.5	22.6 ± 0.7	0.953
ALT, U/L	19.1 ± 0.9	18.1 ± 0.6	19.1 ± 0.7	0.475	16.6 ± 0.9	17.8 ± 0.6	16.4 ± 0.7	0.241
γ-GTP, IU/L	32.6 ± 4.6	35.6 ± 3.1	42.7 ± 3.8	0.194	20.2 ± 1.6	23.4 ± 1.1	22.9 ± 1.3	0.254
Fasting insulin, μU/mL	4.7 ± 0.3	4.3 ± 0.2	4.4 ± 0.2	0.439	4.6 ± 0.3	4.4 ± 0.2	3.9 ± 0.2 a	**0.025**
IRI AUC	5440.1 ± 322.4	5253.7 ± 217.0	5362.4 ± 268.0	0.881	5885.8 ± 292.5	5652.4 ± 189.2	5652.4 ± 189.2	**0.039**
Fasting glucose, mg/dL	97.2 ± 0.9	97.6 ± 0.6	96.4 ± 0.8	0.473	93.2 ± 0.7	93.7 ± 0.5	92.4 ± 0.6	0.173
Glucose AUC	17703.7 ± 368.8	17899.3 ± 248.3	17240.3 ± 306.5	0.248	17027.4 ± 316.1	16830.6 ± 204.5	16719.8 ± 249.1	0.747
Fasting FFAs, μEq/L	466.7 ± 19.9	465.1 ± 13.4	484.9 ± 16.5	0.626	501.5 ± 19.7	531.9 ± 12.7	516.6 ± 15.6	0.411
HOMA-IR	1.2 ± 0.07	1.1 ± 0.05	1.1 ± 0.06	0.444	1.1 ± 0.06	1.0 ± 0.04	0.9 ± 0.05 a	**0.020**
HOMA-β, %	49.4 ± 2.7	45.0 ± 1.8	47.4 ± 2.2	0.367	55.0 ± 2.9	52.2 ± 1.9	47.8 ± 2.3	0.122
Matsuda index	7.7 ± 0.4	8.1 ± 0.3	8.3 ± 0.4	0.550	7.8 ± 0.3	7.9 ± 0.2	8.7 ± 0.3 a, b	**0.019**

Data are expressed as mean ± SD. Adjusted variables: Age, BMI, PA. *p* value, Bold values indicate statistically significant differences ($p < 0.05$) followed by ANCOVA for continuous variables.
$p < 0.05$, a versus C/C for Bonferroni post hoc test.
$p < 0.05$, b versus C/T for Bonferroni post hoc test.
ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; FFA, free fatty acid; FGF21, fibroblast growth factor 21; GCKR, glucokinase regulatory protein; HOMA-IR, homeostasis model assessment of insulin resistance index; HOMA-β, homeostasis model assessment of beta cell function; HsCRP, high-sensitivity C-reactive protein; IRI, immunoreactive insulin; PA, physical activity; TG, triglycerides; γ-GTP, gamma glutamyl transferase.

On the other hand, previous studies[43–45] have shown that FGF21 influences body weight regulation. While our study did not directly assess the mediation effect of FGF21, the observed relationships and existing knowledge suggest that FGF21 may mediate the effects of the GCKR polymorphism on body weight. Further research, including interventional studies, is necessary to confirm these findings, and this is considered an important area for future investigation.

Genome-wide association studies and candidate gene studies have identified Single Nucleotide Polymorphism (SNP) in *GCKR* that is strongly associated with a range of traits. In particular, there is very strong evidence that the T allele of rs1260326 is associated with increased levels of TG[15,19] and CRP,[16,17] and lower glucose levels.[12–14,19,20,46] Consistent with these previous studies, this study found that the T allele of rs1260326 was associated with increased insulin sensitivity and higher CRP levels, but not with TG. Previous studies have suggested a weaker association of the rs1260326 T allele with serum TG in individuals with NGT compared with those with type 2 diabetes.[47] This might explain the lack of this association in our study, which predominantly included subjects with NGT. Furthermore, contradictory results were found in women with the GCKR rs1260326 T/T genotype, where elevated CRP levels were observed despite high insulin sensitivity. Since the GCKR rs1260326 genotype has also been identified as a causative gene for blood CRP levels in genome-wide association studies,[17] the pleiotropic effects of the GCKR gene may affect the trait through different pathways. Thus, the rs1260326 T allele may affect glucose metabolism and inflammatory processes simultaneously through different mechanisms.

FGF21 improves insulin sensitivity and the metabolism of both glucose and lipids.[48,49] This study showed that the *GCKR* rs1260326 T allele was associated with increased serum levels of FGF21.[50] Together these findings suggest that *GCKR* genotype might affect metabolism-related phenotypes through FGF21, a hypothesis that is consistent with our results showing an association between increased FGF21 levels and high insulin sensitivity in women who have the *GCKR* rs1260326 T/T genotype. It was also previously found that exogenous FGF21 treatment decreased carbohydrate intake[51] and increased adiponectin secretion from adipocytes,[52] leading to improved insulin resistance; however, this study found no association between genotype and either dietary intake or adiponectin. Therefore, our findings suggest that the *GCKR* rs1260326 T allele may contribute to enhanced insulin sensitivity, potentially through a direct action of increased FGF21 levels, a hypothesis supported particularly in the context of women.

This study has several limitations. This cross-sectional study in Tokyo investigated the effect of the *GCKR* rs1260326 polymorphism on metabolic traits in an older Japanese population. However, caution should be exercised when generalizing the findings due to the specific population and cross-sectional design of the study. Limited causal inference is acknowledged, highlighting the need for longitudinal studies to establish causal relationships. Another limitation of this study is the absence of a power analysis for sample size calculation. The sample size was determined based on practical considerations rather than statistical calculations, which may impact the generalizability and robustness of our findings. Future studies should incorporate power analysis to ensure an adequately powered sample size. In addition, the reliance on self-reported dietary intake introduces potential limitations such as recall bias.

Conclusion

Our study revealed that the *GCKR* rs1260326 polymorphism impacted metabolic-related parameters in a sex-dependent manner in an older Japanese population. Notably, it affected body composition in men and PA in women, with the T allele being particularly associated with improved insulin sensitivity markers in women. These findings highlight the importance of considering sex differences in genetic studies on metabolic health.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of Juntendo University in November 2015 (Nos. 2015078, 2016138, 2016038, 2017121, and 2019085: Supplemental Appendix A). This study was carried out in accordance with the principles outlined in the Declaration of Helsinki. All participants provided written informed consent prior to their inclusion in the study. The consent process ensured that participants were fully aware of the study purpose, procedures, potential risks, and benefits.

Consent for publication

All participants or their legal guardians provided written informed consent for publication.

Author contributions

Shota Sakamoto: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

Saori Kakehi: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing – original draft; Writing – review & editing.

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Naoaki Ito: Data curation; Formal analysis; Investigation.

Ryuzo Kawamori: Investigation.

Hirotaka Watada: Investigation; Writing – review & editing.

Yoshifumi Tamura: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Writing – original draft; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The International Physical Activity Questionnaire (IPAQ) used in this study is available at the official IPAQ website (https://sites.google.com/view/ipaq/home; Supplemental Appendix B). The BDHQ used in this study is available on the official website of the Department of Social and Preventive Epidemiology. School of Public Health, Division of health science ant nursing, Graduate School of Medicine, the university of Tokyo (Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo (https://www.nebn.m.u-tokyo.ac.jp/), and applications can be made through the contact form on this website.

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Supplemental material

Supplemental material for this article is available online.

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