Table 4. Cases with recurrence after ER for submucosal CRC

No.	Gender	Age	Location	Macroscopic type	Size (mm)	ER technique	Vertical margin	Lateral margin	Depth of SM (μm)	Lymphatic/ venous involvement	Histological grade	Budding	Local recurrence	Metastatic recurrence	Alive/ death	Interval after ER (month)
1	F	68	C	Protruded	≧20	Piecemeal	?	+	240	−	well	−	IM	+	Alive	4
2	M	68	S	Superficial	≧20	Piecemeal	+	?	250	+	mod	−	SM	+	Alive	15
3	M	62	S	Protruded	<20	En bloc	−	+	SM scanty	?	well	−	IM	−	Alive	14
4	M	63	S	Protruded	≧20	En bloc	−	−	1000	+	mod	+	SM	+	Death	16
5	F	69	R	Superficial	<20	En bloc	−	−	1024	−	well	−	?	+	Alive	14
6	M	73	R	Protruded	<20	En bloc	−	−	1300	−	mod	−	SM	+	Death	20
7	F	60	S	Protruded	<20	En bloc	−	−	1572	−	well	−	SM	+	Alive	89
8	M	61	R	Protruded	≧20	En bloc	+	−	1800	+	mod	−	?	+	Death	12
9	F	71	A	?	≧20	Piecemeal	−	−	2200	+	mod	+	SM	+	Alive	24
10	F	78	C	Superficial	<20	Piecemeal	+	?	2433	−	well	−	SM	−	Alive	16
11	M	59	A	Protruded	<20	En bloc	−	−	3000	+	por	+	?	+	Death	18
12	F	80	R	Protruded	<20	En bloc	−	−	3500	−	well	−	SM	+	Alive	60
13	M	68	S	Protruded	≧20	En bloc	?	?	3800	+	mod	+	IM	−	Alive	8
14	M	74	S	Protruded	<20	En bloc	−	−	4200	+	mod	−	SM	+	Death	0
15	M	66	R	Protruded	<20	En bloc	?	−	5300	−	well	+	SM	−	Death	26
16	M	65	R	Protruded	≧20	En bloc	−	−	6886	+	mod	+	SM	+	Death	22
17	M	81	R	Protruded	<20	En bloc	+	−	SM3	−	?	−	SM	−	Death	10
18	F	80	R	Superficial	≧20	En bloc	?	+	?	−	?	−	IM	+	Alive	10

A, ascending colon; C, cecum; F, female; IM, intramucosa; M, male; mod, moderately differentiated adenocarcinoma; por, poorly differentiated adenocarcinoma; R, rectum; S, sigmoid colon; SM, submucosa; well, well-differentiated adenocarcinoma.

Table 5. Relationship between the depth of submucosal invasion and positive rate of vertical margin in each macroscopic type of submucosal CRC

Depth of submucosal invasion (μm)	Macroscopic type	
	Protruded type $n = 286$	Superficial type $n = 82$
~1000	7.1% (11/156)	2.9% (2/68)
1001~2000	10.2% (6/59)	8.3% (1/12)
2001~3000	14.7% (5/34)	0% (0/7)
3001~4000	18.8% (3/16)	
4001~	9.5% (2/21)	0% (0/2)
Total	9.4% (27/286)	3.7% (3/82)

CRC, colorectal carcinoma.

Table 6. Relationship between vertical margin and recurrence in endoscopically resected submucosal CRC without additional surgical resection

Vertical margin	Recurrence		Total
	Positive	Negative	
Positive	1 (3.2)	31 (96.8)	32 (100)
Negative	9 (2.5)	347 (97.5)	356 (100)

There were no intramucosal recurrent cases.
CRC, colorectal carcinoma.

macroscopic type (protruded or superficial type) in the 368 cases in which the depth of submucosal invasion was reported (Table 5). The overall positive rate of vertical margin was 8.2% (30/368), and the positive rate of protruded and superficial type lesions was 9.4% (27/286) and 3.7% (3/82), respectively. There were no significant differences between each macroscopic type. The positive rate of vertical margin of the protruded and superficial type lesions with submucosal invasion ≦1000 μm was 7.1% (11/156) and 2.9% (2/68), respectively. The positive rate of vertical margin of the protruded and superficial type lesions with submucosal invasion >1000 μm was 6.2% (16/259) and 4.8% (1/21), respectively. With regard to the relationship between vertical margin and recurrence, the recurrence rate for the vertical margin-positive cases was 3.1% (1/32) and that for the vertical margin-negative cases was 2.5% (9/356). There were no significant differences between the values for these two groups (Table 6).

DISCUSSION

This multi-institution questionnaire survey had several limitations. Owing to the retrospective-examination model, the average follow-up period was 38.7 months, and a central review was not carried out on the pathological specimens. However, valuable data were obtained by analyzing the prognoses of non-surgical submucosal CRC cases after ER, which were provided by multiple institutions. Our data showed that all non-surgical submucosal CRC cases with recurrence after

ER did not satisfy the curative conditions according to the Guidelines for Colorectal Cancer Treatment, 1st Edition by JSCCR.[1] As the curative conditions after ER for submucosal CRC stated in the currently used Guidelines for Colorectal Cancer Treatment, 2nd Edition by JSCCR, 2009[5] (a factor of budding grade was added to the curative condition of the 1st Edition) were derived from examinations of the cases in which surgical resection was accompanied by LN metastasis, micrometastasis was not taken into consideration. Clinical verification of the curative conditions can be proved by the prognosis of the non-surgical submucosal CRC cases after ER. However, as these results were obtained on the basis of the histopathological diagnosis in different institutions, we presumed that a certain amount of scattering existed among the data from different institutions.

ER is a therapeutic technique as well as an important diagnostic method that can be used as the total incisional biopsy. Complete resection of the lesions, including vertical margin-negative, is indispensable for curative conditions after ER for submucosal CRC. Currently, among the factors in the curative conditions, only the depth of submucosal invasion can be diagnosed prior to the surgical operation. LN metastasis of submucosal CRC with invasion depth deeper than 1500 μm and 2000 μm was not observed under certain conditions when the histological grade at the deepest invasive portion was taken into consideration.[6] Our data showed that the incidence of the histopathological vertical margin-positive was 8.2%, and there was no significant relationship between the vertical margin-positive and recurrence. This result may show that there is a difference between the histologically vertical margin-positive and submucosal residual tumors on the colorectal wall. To avoid local recurrence after ER, it is important to observe an ulcer in detail using colonoscopy. A previous report has described that residual tumors caused by incomplete ER have a higher growth potential than tumors before ER.[7] Therefore, to avoid a potential disadvantage to the patients, the preoperative diagnosis must be carried out precisely, and an appropriate therapy must be selected.

Regarding surveillance for non-surgical submucosal CRC cases after ER, the results obtained in this study revealed that distant metastasis or death due to primary disease within 3 years after ER was observed in 89% of the patients showing recurrence. Therefore, surveillance must be strictly carried out for 3 years after ER for submucosal CRC with non-curative condition. The examinations conducted in other institutions revealed that in many cases, recurrence took place within 5 years after ER.[8–10] Currently, there is no consensus as to the ideal surveillance method and period after ER for submucosal CRC.

This unstable questionnaire survey had several limitations. However, the current status regarding the mid-term prognosis after ER for submucosal CRC without additional surgical operation in Japan is not available. To investigate curative condition after ER for submucosal CRC and the appropriate interval between surveillances, a long-term prognosis survey from a large number of cases must be analyzed in the near future.

CONCLUSION

This questionnaire survey was conducted in institutions affiliated with the Colorectal Endoscopic Resection Standardization Implementation Working Group in JSCCR. The results obtained by analysis of the prognosis of the non-surgical submucosal CRC cases after ER proved that there is no risk of recurrence, and that surveillance could be carried out without additional surgical resection when the lesions satisfied the curative conditions after ER for submucosal CRC according to the Guidelines for Colorectal Cancer Treatment, 1st Edition by JSCCR,[1] and recurrence was observed within 3 years after ER.

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Original Article: Clinical Investigation

Impact of insulin resistance, insulin and adiponectin on kidney stones in the Japanese population

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Objectives: It has been reported that kidney stones are linked to metabolic syndrome (MetS), which is characterized by insulin resistance. The aim of the present study was to examine the association of insulin resistance, insulin and adiponectin with kidney stones in a Japanese population.

Methods: From February 2007 to March 2008, 1036 (529 men and 507 women) apparently healthy Japanese subjects, aged 35–79 years, were analyzed. Weight, height, waist circumference and blood pressure were measured. Overnight fasting blood was collected to measure insulin and adiponectin levels. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to assess insulin resistance. Logistic regression analysis was used to estimate the odds ratio (OR) and 95% confidence intervals for a self-reported history of kidney stones across tertiles of HOMA-IR, insulin and adiponectin.

Results: Of the participants, 84 men (15.6%) and 35 women (6.9%) had a history of kidney stones. Age, body mass index, waist circumference, systolic and diastolic blood pressures, HOMA-IR and insulin were significantly higher in women with than in women without kidney stones. There was no difference in adiponectin level between subjects with and without a history of kidney stones in either sex. Furthermore, a significant positive trend was observed in the age-adjusted OR for a history of kidney stones across insulin tertiles (*P*-value for trend = 0.04) in women.

Conclusions: For Japanese women, HOMA-IR and insulin are associated with a history of kidney stones. The findings suggest that MetS components could increase the risk of kidney stones through subclinical hyperinsulinemia and insulin resistance.

Key words: adiponectin, insulin, insulin resistance, kidney stone, metabolic syndrome.

Introduction

Kidney stones are a common urological problem with a lifetime prevalence of approximately 10% in men and 5–6% in women, and their prevalence has been increasing in many developed countries.[1–3] In Japan, as in other countries, the age-standardized annual incidence of first-episode upper urinary tract stones was 81.3 per 100 000 men and 29.5 per 100 000 women in 1965, and it rose steadily to reach 165.1 per 100 000 men and 65.1 per 100 000 women in 2005.[4] Around the same period, a rapid increase in the prevalence of obesity in Asian countries – including Japan, where obesity traditionally had not been common – was reported.[5] Furthermore, 24.4% of middle-aged Japanese men and 12.1% of middle-aged Japanese women appeared to have metabolic syndrome (MetS) in 2006.[6]

Kidney stone disease has been linked to the major MetS components, including obesity, hypertension and diabetes mellitus (DM), in several epidemiological studies.[7–10] A positive relationship between a self-reported history of kidney stones and the number of MetS components has also been observed in a cross-sectional analysis.[11] MetS is characterized by high insulin resistance and compensatory hyperinsulinemia.[12] Based on this evidence, high insulin resistance and hyperinsulinemia might raise the risk for kidney stone formation. In contrast, adiponectin might protect against the development of kidney stones, because it is the most abundantly circulating adipokine showing an inverse association with adiposity and body mass index (BMI).[13]

Given the rapid increase in the prevalence or incidence of kidney stones and the prevalence of MetS in many nations, it is important to clarify the relationships between kidney stones and MetS. However, the associations of insulin resistance, insulin and adiponectin with kidney stones have not

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been reported. In the present study, associations of MetS components, insulin resistance assessed by homeostasis model assessment of insulin resistance (HOMA-IR), insulin and adiponectin with a self-reported history of kidney stones were investigated in an apparently healthy Japanese population.

Methods

Study population

All procedures of the present study were approved by the Nagoya City University Institutional Review Board. Okazaki City, which is located in the central area of Japan, had a population of 374 358 (189 200 men and 185 158 women) on 1 January 2008. From February 2007 to March 2008, 38 920 people (21 717 men and 17 203 women) visited the Okazaki City Medical Association Public Health Center for a comprehensive health examination. A consent form and a questionnaire for the present study were randomly sent to 3852 inhabitants aged 35–79 years before their medical check-up. An answer to the questionnaire survey was received from 1112 respondents (566 men and 546 women). Participants who did not complete the questions on medical history of kidney stone disease (4 men and 2 women) and those who did not undergo blood examinations (33 men and 37 women) were excluded. The remaining 1036 participants (529 men and 507 women) were analyzed in the present study. Written informed consent was obtained from all participants.

Anthropometrics and biochemistry

Bodyweight (kg), height (cm) and waist circumference (cm) were measured to the nearest 0.1 kg and 0.1 cm, respectively. BMI was calculated as the weight in kilograms/height in meters squared. Blood pressure (BP; mmHg) was measured in the morning after 10 min of rest in the sitting position. Overnight fasting venous blood samples were collected in the morning. After serum was separated, glucose, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol and uric acid levels were immediately measured with a Hitachi autoanalyzer model 7700 (Hitachi Medical, Tokyo, Japan). Serum insulin was measured by a chemiluminescence enzyme immunoassay kit (FUJIREBIO, Tokyo, Japan). Blood was collected in plasma tubes and stored at −80°C until analysis of adiponectin levels. The concentrations of plasma adiponectin were measured using commercially available ELISA kits (Otsuka Pharmaceutical, Tokyo, Japan). HOMA-IR[14] was used to assess insulin resistance. The HOMA-IR value was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L) / 405.[14]

Components of MetS were diagnosed using the Evaluate Diagnostic Standards for MetS in Japan,[15] and participants were classified as patients with MetS if they had central obesity (waist circumference ≥85 cm in men, ≥90 cm in women) and two or more of the three following components: (i) high BP (≥130 mmHg systolic and/or ≥85 mmHg diastolic or self-reported antihypertensive medication use); (ii) high triglyceride level (≥150 mg/dL or self-reported anti-high triglyceride medication use) or low HDL-cholesterol (<40 mg/dL or self-reported anti-low HDL-cholesterol medication use); and (iii) fasting hyperglycemia (≥110 mg/dL or self-reported diabetic medication use).

Questionnaire

The subjects' medical histories were obtained by a self-administered questionnaire that was partially supported and reconfirmed by a personal interview with a trained examiner. Participants who responded "yes" to the question "Have you ever had kidney stone disease?" were defined as having had an episode of kidney stones. Age at first kidney stone or family history of kidney stones was not ascertained. History of and medication for hypertension, DM and dyslipidemia were also self-reported. In women, menopausal status was also checked.

Statistical analysis

All data were analyzed by sex. The demographic variables were calculated and tabulated between subjects with and without a self-reported history of kidney stones. Categorical variables were compared using the χ^2-test or Fisher's exact test, and mean values of continuous variables were compared between groups using Student's *t*-test. Pearson's correlation coefficients were calculated between two variables. Based on HOMA-IR, insulin and adiponectin, participants were grouped into tertiles. The Mantel–Haenszel χ^2-test was used to check trends in the prevalence of self-reported history of kidney stones across HOMA-IR, insulin and adiponectin tertiles. Age-adjusted odds ratios (OR) and 95% confidence intervals (CI) for a self-reported history of kidney stones across HOMA-IR, insulin and adiponectin tertiles were estimated using logistic regression analysis. To test linear trends across HOMA-IR, insulin and adiponectin tertiles, the median of each tertile was used as a continuous variable. Because the distributions of HOMA-IR, insulin and adiponectin were skewed, log-transformed HOMA-IR, insulin and adiponectin were used and subsequently back-transformed for interpretation of the results. All statistical analyses were carried out using the Statistical Analysis System, version 9.1 (SAS Institute, Cary, NC, USA), and significance was defined as $P < 0.05$.

Results

Of the participants, 84 men (15.6%) and 35 women (6.9%) had a history of kidney stones. In men, there were no

Table 1 Subjects' characteristics between men with/without self-reported history of kidney stones

Men ($n = 529$)	Self-reported history of kidney stones		P*
	With ($n = 84$)	Without ($n = 445$)	
Age, mean ± SD (year)	61.2 ± 9.5	60.2 ± 10.4	0.44
Height, mean ± SD (cm)	165.4 ± 5.8	166.0 ± 6.2	0.47
Weight, mean ± SD (kg)	63.7 ± 9.1	64.1 ± 9.8	0.70
BMI, mean ± SD (kg/m^2)	23.2 ± 2.9	23.2 ± 3.0	0.99
Waist circumference, mean ± SD (cm)	84.5 ± 7.6	84.4 ± 7.8	0.91
Systolic BP, mean ± SD (mmHg)	129.8 ± 15.1	128.2 ± 15.2	0.38
Diastolic BP, mean ± SD (mmHg)	81.4 ± 9.5	80.3 ± 9.1	0.32
Total cholesterol, mean ± SD (mg/dL)	202.8 ± 32.2	206.0 ± 30.8	0.39
Triglycerides, mean ± SD (mg/dL)	115.7 ± 69.2	122.3 ± 85.5	0.44
HDL-cholesterol, mean ± SD (mg/dL)	61.4 ± 15.0	64.1 ± 16.9	0.16
Uric acid, mean ± SD (mg/dL)	6.1 ± 1.1	5.9 ± 1.2	0.41
Glucose, mean ± SD (mg/dL)	99.5 ± 16.5	100.3 ± 20.8	0.70
HOMA-IR†	1.14	1.12	0.86
Insulin (μU/mL)†	4.69	4.61	0.79
Adiponectin (μg/mL)†	5.69	5.87	0.62
Overweight ($25 \leq$ BMI < 30), n (%)	18 (21.4)	88 (19.8)	0.73
Obese ($30 \leq$ BMI), n (%)	1 (1.2)	7 (1.6)	1.00
Hypertension, n (%)	25 (29.8)	99 (22.3)	0.14
Diabetes mellitus, n (%)	9 (10.7)	31 (7.0)	0.23
Dyslipidemia, n (%)	14 (16.7)	51 (11.5)	0.18
Metabolic syndrome, n (%)	15 (17.9)	69 (15.5)	0.59

*P-value for t-test, χ^2-test or Fisher's exact test. †Geometric mean. BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; SD, standard deviation.

significant differences between subjects with and without a history of kidney stones (Table 1). In contrast to men, age, BMI, waist circumference, systolic and diastolic BP, HOMA-IR and insulin were significantly higher in women with than in women without a history of kidney stones (Table 2). On the other hand, adiponectin was no different between the two groups of either sex (Tables 1,2). The prevalence of overweight, obese, hypertension, DM, dyslipidemia or MetS was no different between the two groups in men (Table 1). In contrast to men, the prevalence of obesity and MetS in women tended to be higher in the kidney stone group ($P = 0.06$ and 0.07, respectively: Table 2). Postmenopausal status was significantly more frequent in women with a history of kidney stones ($P = 0.02$: Table 2).

Figure 1 shows scatter plots with the r-value for correlation coefficients between natural logarithm (ln) HOMA-IR, ln (insulin), ln (adiponectin) and age (Fig. 1a–c), BMI (Fig. 1d–f), waist circumference (Fig. 1g–i) and systolic BP (Fig. 1j–l) by sex. In both sexes, HOMA-IR and insulin were correlated positively with BMI, waist circumference and systolic BP. In contrast, adiponectin was correlated negatively with BMI and waist circumference in both sexes. The r-values for correlation coefficients between ln (HOMA-IR), ln (insulin), ln (adiponectin), and age (Fig. 1a–c), ln (adiponectin) and systolic BP (Fig. 1l) were low.

In each of the HOMA-IR tertiles, the prevalence of self-reported history of kidney stones and age-adjusted OR (95% CI) are shown in Table 3. In men, the prevalence and age-adjusted OR for history of kidney stones were not different across HOMA-IR tertiles. Although upper HOMA-IR tertiles had the highest prevalence of self-reported history of kidney stones and the highest age-adjusted OR in women, no significant trend was found (P-value for trend = 0.09 and 0.14, respectively: Table 3 & Fig. 2a). Table 4 shows the prevalence of self-reported history of kidney stones and age-adjusted OR (95% CI) across insulin tertiles. In women, significant positive trends were observed for the prevalence of self-reported history of kidney stones and age-adjusted OR across insulin tertiles (P-value for trend = 0.03 and 0.04, respectively: Table 4 & Fig. 2b). In each of the adiponectin tertiles, no significant trends were observed for the prevalence and age-adjusted OR for a history of kidney stones in either sex (Table 5 & Fig. 2c).

Table 2 Subjects' characteristics between women with/without self-reported history of kidney stones

Women ($n = 507$)	Self-reported history of kidney stones		P*
	with ($n = 35$)	without ($n = 472$)	
Age, mean ± SD (year)	59.3 ± 6.9	56.0 ± 9.5	0.01
Height, mean ± SD (cm)	154.4 ± 5.3	154.2 ± 5.3	0.83
Weight, mean ± SD (kg)	56.4 ± 11.2	52.7 ± 7.3	0.06
BMI, mean ± SD (kg/m^2)	23.6 ± 3.8	22.1 ± 2.8	0.04
Waist circumference, mean ± SD (cm)	85.6 ± 10.8	81.4 ± 8.0	0.03
Systolic BP, mean ± SD (mmHg)	131.3 ± 16.3	122.7 ± 15.6	0.002
Diastolic BP, mean ± SD (mmHg)	80.0 ± 9.0	76.4 ± 9.3	0.03
Total cholesterol, mean ± SD (mg/dL)	221.0 ± 30.6	215.6 ± 33.6	0.36
Triglyceride, mean ± SD (mg/dL)	102.6 ± 49.0	92.7 ± 51.8	0.27
HDL cholesterol, mean ± SD (mg/dL)	74.5 ± 19.8	76.9 ± 17.4	0.44
Uric acid, mean ± SD (mg/dL)	4.7 ± 1.0	4.4 ± 0.9	0.13
Glucose, mean ± SD (mg/dL)	95.7 ± 13.6	93.9 ± 12.7	0.42
HOMA-IR†	1.31	1.08	0.04
Insulin (μU/mL)†	5.60	4.71	0.04
Adiponectin (μg/mL)†	8.57	9.43	0.23
Overweight ($25 \leq$ BMI < 30), n (%)	8 (22.9)	65 (13.8)	0.14
Obese ($30 \leq$ BMI), n (%)	2 (5.7)	4 (0.8)	0.06
Hypertension, n (%)	7 (20.0)	76 (16.1)	0.55
Diabetes mellitus, n (%)	2 (5.7)	18 (3.8)	0.64
Dyslipidemia, n (%)	7 (20.0)	76 (16.1)	0.55
Metabolic syndrome, n (%)	4 (11.4)	19 (4.0)	0.07
Menopause, n (%)‡	28 (93.3)	327 (73.2)	0.02

*P-value for t-test, χ^2-test or Fisher's exact test. †Geometric mean. ‡Missing values ($n = 30$ in menopausal status) were excluded in the calculation of percentage (%). BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; SD, standard deviation.

Discussion

The present study was carried out to explore associations between MetS components and a history of kidney stones in an apparently healthy Japanese population with a smaller BMI than Caucasians and Africans. Positive associations of obesity estimated by BMI and waist circumference, systolic BP and diastolic BP with a history of kidney stones were found in Japanese women.

Previous epidemiological studies also showed that the effect of obesity, hypertension and DM on kidney stones was present predominantly in women.[8,10,16,17] The Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS) I and II showed that the relative risk for a patient with kidney stones who weighs more than 100.0 kg *vs* a patient with kidney stones who weighs less than 68.2 kg was 1.44 (95% CI, 1.11–1.86) in men, 1.89 (95% CI, 1.52–2.36) in older women and 1.92 (95% CI, 1.59–2.31) in younger women.[8] BMI and waist circumference were also associated with stone formation, predominantly in women rather than in men in the prospective studies.[8] Another study involving approximately 6000 patients with urinary stone disease showed that obesity (men > 120 kg, women > 100 kg) was more prevalent among female patients than male patients (3.8% of men and 12.6% of women); furthermore, the mean number of stone episodes was significantly higher in women over 100 kg than in women under 85 kg (2.93 *vs* 3.38), but not in men.[17] The Third National Health and Nutrition Examination Survey showed that stone formers had a 69% increase in self-reported hypertension compared with non-stone formers among women, but not among men.[16] Diabetes was associated with prevalent stone disease in both sexes and an increased risk of incident kidney stone formation in women in the HPFS and the NHS.[10]

The mechanism of sex differences for the effect of obesity, hypertension and DM on kidney stone disease is uncertain. BMI was associated positively with urinary oxalate excretion in both sexes, but this relationship persisted only in women after adjusting for urinary phosphate and uric acid excretion.[18] In fact, the urinary excretion of lithogenic factors depending on body size was also different in the two sexes. In the present study, MetS components did not differ between male subjects with or without kidney

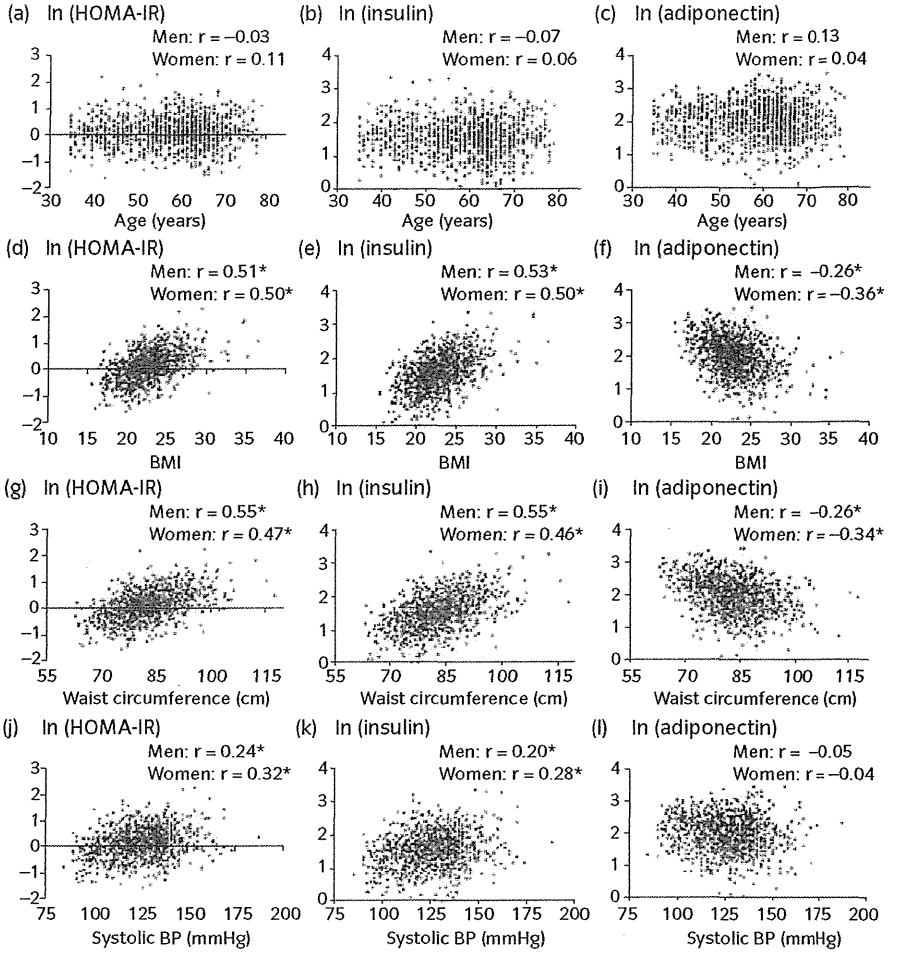

Fig. 1 Scatter plots with the *r*-values for correlation coefficients between (a–c) natural logarithm (ln) homeostasis model assessment of insulin resistance (HOMA-IR), ln (insulin), ln (adiponectin) and age, (d–f) body mass index (BMI), (g–i) waist circumference, and (j–l) systolic blood pressure (BP) by sex. *$P < 0.0001$. (•), Men; (•), women.

Table 3 The prevalence of subjects with self-reported kidney stone and age-adjusted OR (95% CI) across homeostasis model assessment of insulin resistance tertiles

	Tertile			*P* for trend
	Lower	Middle	Upper	
Men ($n = 529$)	$n = 176$	$n = 177$	$n = 176$	
Median HOMA-IR value (range)	0.62 (0.20–0.89)	1.13 (0.90–1.42)	2.03 (1.43–9.91)	
Self-reported kidney stone, *n* (%)	31 (17.6)	28 (15.8)	25 (14.2)	0.38*
Age-adjusted OR (95% CI)	1.00	0.89 (0.51–1.57)	0.78 (0.44–1.39)	0.41**
Women ($n = 507$)	$n = 168$	$n = 170$	$n = 169$	
Median HOMA-IR value (range)	0.64 (0.25–0.91)	1.10 (0.91–1.32)	1.75 (1.33–9.13)	
Self-reported kidney stone, *n* (%)	8 (4.8)	11 (6.5)	16 (9.5)	0.09*
Age-adjusted OR (95% CI)	1.00	1.31 (0.51–3.37)	1.89 (0.78–4.60)	0.14**

*P-values for trend in proportions of subjects with self-reported kidney stone using Mantel–Haenszel chi-square test. **P-values calculated using median of tertiles as a continuous variable in logistic regression models.

stone history, due in part to the small number of subjects. With an increased number of study subjects, the differences might reach significance in men. However, impacts of Mets components on kidney stone formation in men would not be greater than those in women.

Primarily, the basis for sex differences in kidney stone formation is unclear, though kidney stones are more common in men than women. Some hormonal factors might be involved. In the present study, women with a history of kidney stone formation were older than women without a

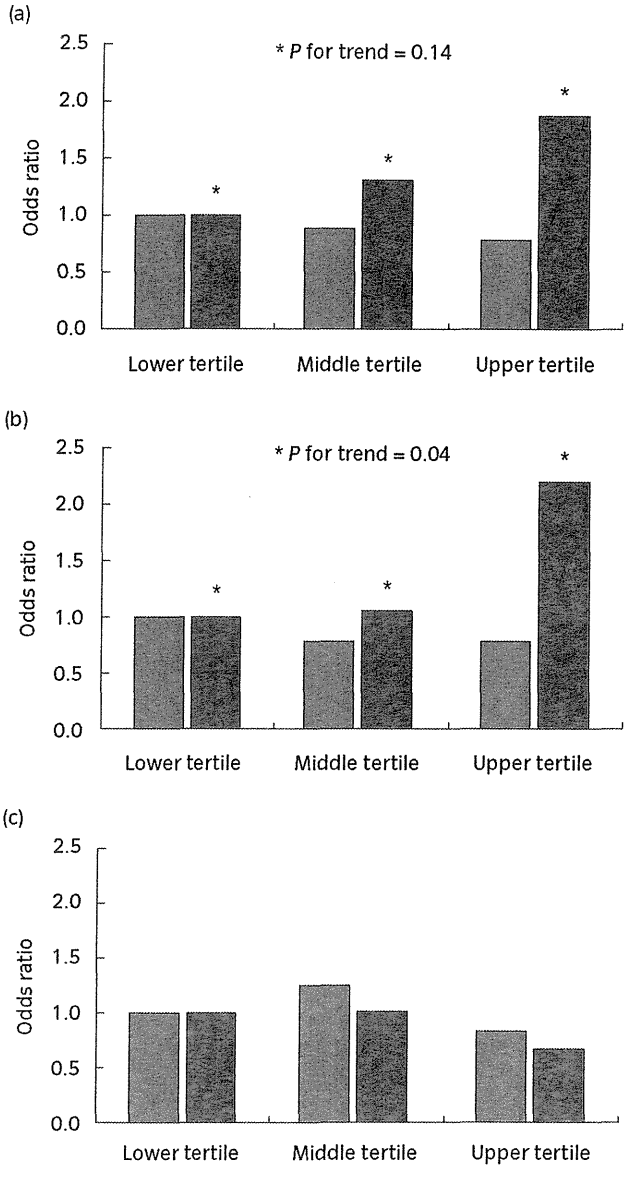

Fig. 2 Age-adjusted odds ratio for a self-reported history of kidney stones across tertiles of (a) homeostasis model assessment of insulin resistance (HOMA-IR), (b) insulin and (c) adiponectin. (▨), Men; (■), women.

history of kidney stone formation, and postmenopausal status was significantly more frequent in women with a history of kidney stone formation. Estrogen, which reduces urinary excretion of calcium, is considered a protective factor for kidney stone formation.[19] Therefore, menopause could be a risk for kidney stone formation by increasing urinary calcium excretion in women.[20,21] However, no independent association was observed between menopause or postmenopausal hormone use and the risk of kidney stone formation.[22] In the present study, data regarding postmenopausal hormone use were lacking; however, only approximately 5% of postmenopausal Japanese women have been reported to take hormone replacement therapy.[23] Because age at the first onset of kidney stone was not surveyed in the present study, we could not examine these issues accurately.

Relationships of insulin resistance as assessed by HOMA-IR and insulin with history of kidney stone formation were also identified in the present study; HOMA-IR and insulin were significantly higher in women with a history of kidney stone formation, but not in men. Furthermore, the age-adjusted OR for history of kidney stone formation showed a positive trend across insulin tertiles (P-value for trend = 0.04: Table 4 & Fig. 2b) in women. Although the age-adjusted OR of women with a self-reported history of kidney stones increased across HOMA-IR tertiles, the P-value for the trend did not reach significance (P-value for trend = 0.14: Table 3 & Fig. 2a). In the present study, the "gold standard" for assessment of insulin resistance, the glucose clamp method, was not used,[24] and diabetic patients were not excluded. HOMA-IR might not be appropriate for assessing insulin resistance in advanced diabetic patients, because HOMA-IR relies on the product of fasting glucose and insulin. Therefore, HOMA-IR as a parameter of insulin resistance could have affected the results of the present study. However, many previous studies have shown that HOMA-IR has a close correlation with the insulin resistance measured using the glucose clamp method in patients with or without DM.[14,25,26]

The mechanism of hyperinsulinemia and insulin resistance on kidney stone formation has been described. High insulin resistance and hyperinsulinemia could contribute to the development of calcium stones by lowering urinary citrate excretion[27] or increasing urinary calcium excretion.[28–30] In both sexes, HOMA-IR and insulin were positively correlated with BMI, waist circumference, and systolic and diastolic BP in the present study (Fig. 1) However, the prevalence of self-reported hypertension, DM and dyslipidemia was not associated with kidney stones in either sex, due in part to the small number of patients.

In contrast, adiponectin was not different between subjects with and without a history of kidney stones in either sex, though we might have expected adiponectin to show a negative relationship with kidney stones. In both sexes, adiponectin was negatively correlated with BMI and waist circumference in the present study (Fig. 1). However, r-values for correlation coefficients between ln (adiponectin) and obesity estimated by BMI and waist circumference were lower than those between ln (HOMA-IR), ln (insulin) and obesity in both sexes (Fig. 1). Therefore, the effect of adiponectin on kidney stones through obesity could not be detected. In the present study, the participants were apparently healthy; larger numbers of subjects with hypoadiponectinemia are needed to correctly evaluate the effect of adiponectin on kidney stone disease.

The limitations of the present study are as follows. First, a history of kidney stones by self-reported questionnaire is

Table 4 The prevalence of subjects with self-reported kidney stone and age-adjusted OR (95% CI) across insulin tertiles

	Tertile			*P* for trend
	Lower	Middle	Upper	
Men (*n* = 529)	*n* = 180	*n* = 171	*n* = 178	
Median insulin (range), μU/mL	2.70 (1.10–3.70)	4.60 (3.80–5.70)	7.90 (5.80–27.50)	
Self-reported kidney stone, *n* (%)	33 (18.3)	25 (14.6)	26 (14.6)	0.33*
Age-adjusted OR (95% CI)	1.00	0.77 (0.44–1.36)	0.77 (0.44–1.36)	0.41**
Women (*n* = 507)	*n* = 167	*n* = 172	*n* = 168	
Median insulin (range), μU/mL	3.00 (1.10–4.00)	4.80 (4.10–5.70)	7.30 (5.80–29.10)	
Self-reported kidney stone, *n* (%)	8 (4.8)	9 (5.2)	18 (10.7)	0.03*
Age-adjusted OR (95% CI)	1.00	1.04 (0.39–2.77)	2.20 (0.92–5.26)	0.04**

**P*-values for trend in proportions of subjects with self-reported kidney stone using Mantel-Haenszel χ^2-test. ***P*-values calculated using median of tertiles as a continuous variable in logistic regression models.

Table 5 The prevalence of subjects with self-reported kidney stone and age-adjusted OR (95% CI) across adiponectin tertiles

	Tertile			*P* for trend
	Lower	Middle	Upper	
Men (*n* = 529)	*n* = 176	*n* = 177	*n* = 176	
Median adiponectin (range), μg/mL	3.70 (1.07–4.68)	5.83 (4.71–7.01)	9.56 (7.05–31.80)	
Self-reported kidney stone, *n* (%)	27 (15.3)	33 (18.6)	24 (13.6)	0.66*
Age-adjusted OR (95% CI)	1.00	1.25 (0.71–2.19)	0.85 (0.47–1.55)	0.48**
Women (*n* = 507)	*n* = 169	*n* = 171	*n* = 167	
Median adiponectin (range), μg/mL	6.08 (2.09–8.01)	9.57 (8.03–11.70)	14.40 (11.80–31.90)	
Self-reported kidney stone, *n* (%)	13 (7.7)	13 (7.6)	9 (5.4)	0.41*
Age-adjusted OR (95% CI)	1.00	1.03 (0.46–2.31)	0.67 (0.28–1.62)	0.36**

**P*-values for trend in proportions of subjects with self-reported kidney stone using Mantel–Haenszel χ^2-test. ***P*-values calculated using median of tertiles as a continuous variable in logistic regression models.

susceptible to recall bias. However, a self-reported history of kidney stones was validated in a previous study.[31] Second, the present study was limited by several absent data. Kidney stones form in response to environmental and/or metabolic risk factors, such as diet and fluid intake. The traditional Japanese diet has changed remarkably to a Western-style diet rich in animal fat and dairy foods in the post-World War II era.[32] Therefore, diet is an essential variable to investigate the risk for kidney stones in a prospective study. Because the present study was cross-sectional, adjustment for dietary factors that might alter the development of kidney stones could not be carried out. Data on kidney stone composition were also lacking; however, both calcium oxalate and calcium phosphate stones accounted for over 90% of all kidney stones in Japanese patients in 2005.[4] No urinalysis data were available. Thus, whether HOMA-IR, insulin and adiponectin affect urinary pH and calcium excretion was not determined in the present study.

In Japanese women, a history of kidney stones was positively associated with obesity, high BP, HOMA-IR and insulin, and there was a significant positive trend in age-adjusted OR for a history of kidney stones across insulin tertiles. The present result suggests that MetS components could increase the risk of kidney stones through subclinical hyperinsulinemia or insulin resistance in Japanese women. Because of the cross-sectional design of the present study, the risk of hyperinsulinemia and insulin resistance for kidney stone formation needs to be confirmed in a prospective study.

Acknowledgments

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Associations of *apolipoprotein A5 (APOA5), glucokinase (GCK)* and *glucokinase regulatory protein (GCKR)* polymorphisms and lifestyle factors with the risk of dyslipidemia and dysglycemia in Japanese – a cross-sectional data from the J-MICC Study

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Abstract. This study examined the associations of the *APOA5* T-1131C (rs662799), G553T (Cys185Gly, rs2075291), *GCK* G-30A (rs1799884), *GCKR* A/G at intron 16 (rs780094) and T1403C (Leu446Pro, rs1260326) polymorphisms with serum lipid and glucose levels in Japanese, considering lifestyle factors. Study subjects were 2,191 participants (aged 35-69 years, 1,159 males) enrolled in the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study. Dyslipidemia was defined as fasting serum triglycerides (FTG) ≥ 150 mg/dl and/or HDL-cholesterol (HDL-C) < 40 mg/dl, while dysglycemia was as fasting blood sugar (FBS) ≥ 110 mg/dl. When those with *APOA5* -1131 *T/T* or 553 *G/G* were defined as references, those with *APOA5* -1131 *T/C, C/C* or 553 *G/T, T/T* demonstrated significantly elevated risk of dyslipidemia (age- and sex-adjusted odds ratio: 1.77 [95% confidence interval:1.39-2.27], 3.35 [2.41-4.65], 2.23 [1.64-3.02] and 13.78 [3.44-55.18], respectively). Evaluation of FTG, HDL-C or FBS levels according to the genotype revealed that FTG and HDL-C levels were significantly associated with the *APOA5* T-1131C and G553T polymorphisms, FTG with the *GCKR* rs780094 and rs1260326 polymorphisms, and FBS with the *GCKR* rs780094 and rs1260326 polymorphisms. Moreover, a significant positive interaction between *APOA5* 553 *G/T+T/T* genotypes and fat intake ≥ 25% of total energy for the risk of dyslipidemia was observed. Our cross-sectional study confirmed the essential roles of the polymorphisms of the *APOA5*, *GCK* and *GCKR* in the lipid or glucose metabolism disorders, and suggested the importance of fat intake control in the individualized prevention of dyslipidemia.

Key words: *APOA5, GCK(R),* Single nucleotide polymorphisms, Dyslipidemia, Dysglycemia

IN THE RECENT DECADES, sedentary lifestyles became more and more common, and lifestyle-related diseases including coronary heart disease (CHD) or cerebrovascular diseases still remain as one of the biggest threats of deaths for humans both in developed and developing countries. Numbers of studies established that disruptions in the controls of lipid profiles and blood glucose levels increase the risk of these diseases [1, 2].

Recent genome-wide association studies (GWAS) revealed that *GCK* and *GCKR* are potential loci for

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modulating serum triglyceride or fasting serum glucose levels [3]. Among these loci, *GCK* G-30A (rs1799884), *GCKR* A/G at intron 16 (rs780094) and T1403C (Leu446Pro, rs1260326) polymorphisms have been well investigated [4, 5]. The association between polymorphisms in GCK and GCKR genes with the risk of type II diabetes is also reported [6, 7]. Meanwhile, two single nucleotide polymorphisms (SNPs) in the *APOA5* gene, *APOA5* T-1131C (rs662799) or *APOA5* G553T (rs2075291) polymorphisms, are shown to modulate the lipid profiles in Japanese or Chinese subjects [8-10]. While a considerable number of studies have demonstrated significant changes in lipid profiles according to the *APOA5* T-1131C genotype, however, there are still few reports about the influence of *APOA5* G553T polymorphism on the lipid profiles or blood glucose levels. Besides, there exist no studies ever that investigated the gene-environment interaction between these polymorphisms in *APOA5*, *GCK* and *GCKR* genes and lifestyle factors on the risk of lipid or glucose metabolism disorders.

In 2005, we launched the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study, a large genome cohort study to confirm and detect gene-environment interactions in lifestyle related diseases, mainly cancer, which is supported by a research grant for Scientific Research on Special Priority Areas of Cancer from the Japanese Ministry of Education, Culture, Sports, Science and Technology [11].

In this study, we examined the associations of the polymorphism in *GCK, GCKR* and *APOA5* genes with the lipid profiles and blood glucose levels, considering lifestyle factors, in a large Japanese sample using the cross-sectional data of the J-MICC Study.

Subjects and Methods

Study subjects

Subjects were participants of J-MICC Study, in which voluntarily enrolled participants aged 35-69 years from 10 areas of Japan provided their blood and their lifestyle data based on the questionnaire after informed consent [11]. The study is expected to enroll 100,000 participants throughout Japan and follow up the participants until the year 2025. Among the 60,000 participants already enrolled, 4,519 subjects were arbitrarily selected, which consisted of about 500 subjects from each area, and genotyping of 108 selected polymorphisms were conducted for these subjects [12]. From these 4,519 subjects, 2,120 were excluded due to lack of serum laboratory data, 332 were due to taking meals within 6 hours before the blood drawings, and 37 were due to the implausible values of estimated energy intake (< 1,000 kcal/day or > 4,000 kcal/day), leaving 2,030 subjects eligible for the analyses. Informed consent was obtained from all the subjects and the study protocol was approved by the Ethics Committees of Nagoya University School of Medicine and the other participating institutions.

Samples and diagnostic criteria

The fasting serum triglycerides (FTG), HDL-cholesterol (HDL-C) and fasting blood sugar (FBS) levels were measured using their serum samples, which was routinely conducted as health check-ups or done for research in participating institutions. The diagnostic criterion for dyslipidemia is FTG $\geq$ 150 mg/dl and/or HDL-C < 40 mg/dl, and that for dysglycemia is FBS $\geq$ 110 mg/dl. We adopted these criteria based on the metabolic syndrome criteria defined by Japan Society for the Study of Obesity (JASSO) [13], because it is widely and practically used in Japan, and has been shown to be predictable of CHD as other world's representative criteria like NCEP-ATP III, AHA/NHLBI or IDF criteria [14].

Evaluation of lifestyle exposure

Lifestyle exposures were evaluated with a self-administered questionnaire checked by trained staffs. The questionnaire included items on smoking status, alcohol consumption and food consumption. Smoking status was classified as current, former or never, and level of exposure was evaluated in pack-years. Former smokers were defined as people who had quitted smoking for at least 1 year. Alcohol consumption of each type of beverage was determined by average number of drinks per day, and then converted into the Japanese sake unit; 'gou' (180 ml), which is equivalent to 23g of ethanol. Intakes of energy and macronutrients were estimated based on responses to a food frequency questionnaire (FFQ), whose validity and reproducibility to estimate nutrient intakes have been well tested and confirmed [15-18]. The correlation coefficients between the FFQ and 3-day food records were 0.49 for energy, 0.61 for %energy from fat and 0.86 for %energy from carbohydrate in men. The corresponding figures in women were 0.44, 0.48 and 0.66 [16].

Genotyping of polymorphisms

DNA was extracted from buffy coat with a BioRobot M48 Workstation (QIAGEN Group, Tokyo). The genotyping of *APOA5*, *GCK* and *GCKR* polymorphisms was conducted by the RIKEN institute using multiplex polymerase chain reaction-based Invader assay (Third Wave Technologies, Madison, WI) as described previously [19]. The genotype distributions of all the 108 polymorphisms examined in this cross-sectional study are shown in the recently published data [12].

Statistical analysis

Logistic regression analysis was performed for estimating age- and sex-adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for dyslipidemia or dysglycemia by genotype. Gene-environment interactions were assessed by the logistic model, which included a multiplicative interaction term as well as variables for each genotype, age, sex, and smoking and drinking habits. The average levels of lipid profiles (FTG and HDL-C) and FBS according to genotype were presented as means ± standard deviations (SD). The levels of lipid profiles and FBS according to genotype were tested by the analysis of covariance (ANCOVA) adjusting for age, sex, smoking and drinking behaviors. Age adjustments in the analyses were done with ages regarded as continuous variables. Accordance with the Hardy-Weinberg's equilibrium, which indicates an absence of discrepancy between genotype and allele frequencies, was checked using the χ^2 test. Haplotype analysis using genotypes in two loci was calculated by the 'haplologit' command of STATA adjusted for age and sex based on the EM algorhythm [20]. The linkage disequilibrium (LD) between the polymorphisms in two loci (D' and r^2) was estimated by the 'pwld' command of STATA. In assessing gene-environment interaction, indicator variables were used to delineate each single exposure (each of gene and environment) and doubly-exposed (both gene and environment) categories to fit the logistic model. The interaction was calculated as the degree of departure from multiplicity of effects as is represented by the beta-coefficient of the doubly-exposed indicator variable, the exponential form (= ORs for interaction) and the significance level (= *P*-value) of which were described as the main results for it. We adopted the criteria adjusted by Bonferroni's correction for the *P*-values in the analyses conducted under confirmatory contexts (*i.e.*, in calculating the aORs of *APOA5*, *GCK* and *GCKR* polymorphisms [single SNPs] for dyslipidemia and dysglycemia: practically, we adopted the significance levels of *P*-values less than 0.0025 for these analyses which derive from 0.05 / 5 [number of single SNPs] * 2 [homozygous minor & heterozygous] * 2 [dyslipidemia & dysglycemia]); *P*-values less than 0.05 were considered as significant in the other analyses. All the calculations were done using the STATA version 10 (Stata Corp, College Station, TX).

Results

Characteristics of the subjects and allele frequency of the APOA5, GCK and GCKR polymorphisms

The characteristics of the subjects are summarized in Table 1. The mean age ± standard deviation was 55.3 ± 8.9 years, and the females were 45.6% in the whole subjects. The genotype frequencies were 43.4% in *T/T*, 44.2% in *T/C*, and 12.4% in *C/C* for the *APOA5* T-1131C polymorphism, which was in Hardy-Weinberg's equilibrium (*-1131C* allele = 0.345, χ^2 = 0.893, *P* = 0.345), 88.1% in *G/G*, 11.4% in *G/T*, and 0.5% in *T/T* for the *APOA5* G553T polymorphism (in Hardy-Weinberg's equilibrium; *553T* allele = 0.062, χ^2 = 0.736, *P* = 0.391), 68.4% in *G/G*, 28.6% in *A/G*, and 3.0% in *A/A* for the *GCK* G-30A (rs1799884) polymorphism (in Hardy-Weinberg's equilibrium; *-30A* allele = 0.173, χ^2 < 0.001, *P* = 0.983), 29.1% in *A/A*, 49.2% in *A/G*, and 21.8% in *G/G* for the *GCKR* rs780094 A/G polymorphism (in Hardy-Weinberg's equilibrium; *G* allele = 0.464, χ^2 = 0.268, *P* = 0.605), and 29.3% in *T/T*, 49.4% in *T/C*, and 21.3% in *C/C* for the *GCKR* rs1260326 T1403C (Leu446Pro) polymorphism (in Hardy-Weinberg's equilibrium; *1403C* allele = 0.460, χ^2 = 0.059, *P* = 0.807). The allele frequencies were similar to those among the genotyped 4,519 subjects; 0.345 for *APOA5 -1131C*, 0.069 for *APOA5 553T*, 0.189 for *GCK -30A*, 0.449 for *GCKR G*, and 0.445 for *GCKR 1403C* [12].

APOA5, GCK and GCKR polymorphisms, lipid profiles and blood glucose levels

When those with *APOA5* -1131 *T/T* or *APOA5* 553 *G/G* were defined as references, those with *APOA5* -1131 *T/C*, *C/C* or *APOA5* 553 *G/T*, *T/T* demonstrated significantly elevated ORs for dyslipidemia with the aOR of 1.77 (95%CI 1.39-2.27), 3.35 (2.41-4.65), 2.23 (1.64-3.02) and 13.78 (3.44-55.18), respectively. When those with *GCKR* rs780094 *A/A* or *GCKR* rs1260326 *T/T* were defined as references, those with

Table 1 Characteristics of the study subjects.

Characteristics	Male n = 1,105	Female n = 925	Total n = 2,030
Age	55.3 ± 8.9	55.2 ± 8.8	55.3 ± 8.9
Energy intake (kcal/day)	1,935.0 ± 354.1	1,561.8 ± 223.9	1,764.9 ± 354.4
Fat intake (energy%)	19.7 ± 5.3	26.0 ± 6.0	22.6 ± 6.4
Carbohydrate intake (energy%)	57.4 ± 6.6	55.9 ± 5.0	56.7 ± 6.0
Alcohol intake (g/day)	25.3 ± 28.9	3.7 ± 11.8	15.4 ± 25.2
Fasting TG (mg/dl)	129.1 ± 88.7	94.6 ± 58.2	113.4 ± 78.2
HDL-cholesterol (mg/dl)	59.4 ± 15.8	69.1 ± 15.7	63.8 ± 16.5
Fasting blood sugar (mg/dl)	102.0 ± 19.8	95.5 ± 17.7	99.1 ± 19.1
Hb_{A1C} (%)*	5.30 ± 0.61	5.23 ± 0.50	5.27 ± 0.57
Dyslipidemia	313 (28.3%)	118 (12.8%)	431 (21.2%)
Dysglycemia	210 (19.0%)	73 (7.9%)	283 (13.9%)
Smoking			
Never	320 (29.0%)	853 (92.2%)	1,173 (57.8%)
Former	488 (44.2%)	27 (2.9%)	515 (25.4%)
Current	297 (26.9%)	45 (4.9%)	342 (16.8%)

The plus-minus values indicate means ± SDs.
*The data for Hb_{A1c} were available in 1,459 subjects (832 males and 627 females).

GCKR rs780094 *G/G* or *GCKR* rs1260326 *C/C* demonstrated decreased ORs for dyslipidemia with the aOR of 0.64 (95%CI 0.47-0.88) and 0.64 (0.47-0.88), respectively, although the *P*-values were insignificant after adjustment for multiple comparisons (Table 2). Haplotype analysis of the *APOA5* polymorphisms in these two loci (*APOA5* T-1131C and *APOA5* G553T) revealed that the *C-G* haplotype and the *C-T* haplotype was significantly associated with the increased risk of dyslipidemia with the OR of 1.59 (95%CI 1.35-1.87) and 2.66 (2.02-3.50), respectively. We also assessed the LD between these two loci (*APOA5* T-1131C and *APOA5* G553T), which revealed that $D' = 1.00$ and $r^2 = 0.13$. Haplotype analysis of the *GCKR* polymorphisms in these two loci (*GCKR* rs780094 and *GCKR* rs1260326) revealed that *G-C* haplotype was significantly associated with the reduced risk of dyslipidemia with the OR of 0.88 (95%CI 0.81-0.95). We also estimated the LD between these two loci (*GCKR* rs780094 and *GCKR* rs1260326), which revealed that $D' = 0.97$ and $r^2 = 0.92$. As for glucose metabolism, those with *APOA5* -1131 *C/C* demonstrated an elevated OR for dysglycemia with the aOR of 1.52 (95%CI 1.03-2.25) relative to those with *APOA5* -1131 *T/T*, although the *P*-values were insignificant after adjustment for multiple comparisons (Table 3). Evaluation of each component of lipid profiles or blood glucose levels according to the genotypes revealed that the FTG and HDL-C levels were significantly associated with *APOA5* -1131 or 553 polymorphisms ($P < 0.001$ for all, ANCOVA), while the blood glucose levels were significantly associated with the *APOA5* -1131, *GCKR* rs780094 and rs1260326 polymorphisms ($P = 0.006$, $P = 0.019$ and $P = 0.003$, respectively) (Table 4). HbA1c levels were not significantly different according to the genotypes (data not shown). As this study was held in 10 institutions, we also conducted the analyses adjusted for institutions, the results of which were not substantially different from the unadjusted results.

Gene-environment interaction between lifestyle factors and polymorphisms of APOA5, GCK and GCKR

Next we evaluated the gene-environment interactions between *APOA5*, *GCK* and *GCKR* polymorphisms and lifestyle factors including dietary factors and smoking behavior on the risk of dyslipidemia or dysglycemia. Dietary factors consist of intakes of energy, fat, carbohydrate and alcohol. A significant positive interaction on the risk of dyslipidemia was observed between *APOA5* 553 *G/T+T/T* genotypes and fat intake $\geq$ 25% of energy, with the OR for interaction of 3.03 (95% CI 1.59-5.74, $P = 0.001$), while significant negative interactions of *APOA5* -1131 *T/C+C/C* genotypes with intakes of carbohydrate was observed, and a marginally significant negative interaction was observed between *APOA5* 553 *G/T+T/T* genotypes and carbohydrate intake (Table 5). As for *GCK* and *GCKR* polymorphisms, we found a significant positive inter-

Table 2 Adjusted odds ratios (aORs) and 95% confidence intervals (95% CIs) of *APOA5, GCK* and *GCKR* polymorphisms for dyslipidemia.

Genotype	Dyslipidemia	Non-dyslipidemia	aOR*	95% CI*	*P**	aOR†	95% CI†	*P*†
APOA5 rs662799 (T-1131C)								
T/T	132	749	1	referent	-	1	referent	-
T/C	211	687	1.77	1.39-2.27	<0.001	1.86	1.45-2.39	<0.001
C/C	88	163	3.35	2.41-4.65	<0.001	3.51	2.52-4.89	<0.001
T/C + C/C	299	850	2.06	1.63-2.60	<0.001	2.16	1.71-2.74	<0.001
APOA5 rs2075291 (G553T)								
G/G	346	1,443	1	referent	-	1	referent	-
G/T	78	153	2.23	1.64-3.02	<0.001	2.28	1.68-3.11	<0.001
T/T	7	3	13.78	3.44-55.18	<0.001	14.44	3.58-58.35	<0.001
T/G + T/T	85	156	2.41	1.79-3.24	<0.001	2.47	1.83-3.34	<0.001
GCK rs1799884 (G-30A)								
G/G	299	1,089	1	referent	-	1	referent	-
G/A	116	465	0.91	0.72-1.16	0.466	0.93	0.72-1.18	0.538
A/A	16	45	1.27	0.71-2.28	0.421	1.36	0.75-2.47	0.309
GCKR rs780094 (A/G at intron 16)								
A/A	145	445	1	referent	-	1	referent	-
G/A	207	791	0.81	0.63-1.04	0.095	0.82	0.64-1.05	0.116
G/G	79	363	0.64	0.47-0.88	0.005	0.65	0.47-0.88	0.006
GCKR rs1260326 (Leu446Pro, T1403C)								
T/T	146	449	1	referent	-	1	referent	-
T/C	208	795	0.81	0.64-1.04	0.100	0.82	0.64-1.05	0.108
C/C	77	355	0.64	0.47-0.88	0.006	0.64	0.47-0.88	0.006

*Adjusted for age and sex; significance level: $P < 0.0025$ (adjusted for multiple comparisons).
†Adjusted for age, sex, and smoking and drinking behaviors; significance level: $P < 0.0025$.

Table 3 Adjusted odds ratios (aORs) and 95% confidence intervals (95% CIs) of *APOA5, GCK* and *GCKR* polymorphisms for dysglycemia.

Genotype	Dysglycemia	Non-dysglycemia	aOR*	95% CI*	*P**	aOR†	95% CI†	*P*†
APOA5 rs662799 (T-1131C)								
T/T	114	767	1	referent	-	1	referent	-
T/C	126	772	1.11	0.84-1.46	0.479	1.10	0.83-1.45	0.503
C/C	43	208	1.52	1.03-2.25	0.037	1.51	1.02-2.24	0.039
APOA5 rs2075291 (G553T)								
G/G	257	1,532	1	referent	-	1	referent	-
G/T	25	206	0.69	0.44-1.07	0.101	0.68	0.44-1.07	0.094
T/T	1	9	0.92	0.11-7.69	0.941	0.92	0.11-7.65	0.938
GCK rs1799884 (G-30A)								
G/G	193	1,195	1	referent	-	1	referent	-
G/A	80	501	0.96	0.72-1.27	0.756	0.96	0.72-1.28	0.788
A/A	10	51	1.18	0.59-2.37	0.644	1.19	0.59-2.40	0.625
GCKR rs780094 (A/G at intron 16)								
A/A	76	514	1	referent	-	1	referent	-
G/A	136	862	1.11	0.82-1.51	0.491	1.11	0.82-1.51	0.512
G/G	71	371	1.26	0.88-1.79	0.211	1.25	0.87-1.78	0.225
GCKR rs1260326 (Leu446Pro, T1403C)								
T/T	72	523	1	referent	-	1	referent	-
T/C	142	861	1.26	0.93-1.72	0.138	1.26	0.92-1.71	0.148
C/C	69	363	1.35	0.94-1.95	0.102	1.34	0.93-1.93	0.111

*Adjusted for age and sex; significance level: $P < 0.0025$ (adjusted for multiple comparisons).
†Adjusted for age, sex, and smoking and drinking behaviors; significance level: $P < 0.0025$.

Table 4 Lipid profiles and serum glucose levels according to genotypes of *APOA5, GCK* and *GCKR*.

Genotype	*n*	FTG (mg/dl)	*P**	HDL-C (mg/dl)	*P**	FBS (mg/dl)	*P**
APOA5 rs662799 (T-1131C)							
T/T	881	98.4±55.3		65.5±16.3		97.9±16.5	
T/C	898	117.1±79.3	<0.001	63.5±16.7	<0.001	99.6±20.3	0.006
C/C	251	152.7±118.2		58.8±15.0		101.4±22.7	
APOA5 rs2075291 (G553T)							
G/G	1,789	108.9±71.4		64.3±16.4		99.2±19.4	
G/T	231	140.3±102.4	<0.001	60.7±16.6	<0.001	98.5±17.7	0.777
T/T	10	290.3±207.6		49.2±13.6		94.5±10.4	
GCK rs1799884 (G-30A)							
G/G	1,388	114.9±81.6		63.3±16.2		98.8±20.3	
G/A	581	109.2±71.4	0.410	65.1±17.0	0.132	99.4±16.4	0.285
A/A	61	118.1±60.2		63.1±15.2		101.8±16.1	
GCKR rs780094 (A/G at intron 16)							
A/A	590	119.8±75.1		64.0±16.4		97.2±17.4	
G/A	998	111.7±80.0	0.055	64.0±16.5	0.741	99.4±20.8	0.019
G/G	442	108.6±78.0		63.0±16.1		100.6±17.1	
GCKR rs1260326 (Leu446Pro, T1403C)							
T/T	595	119.2±73.5		63.9±16.6		96.9±16.0	
T/C	1,003	112.2±80.7	0.060	64.1±16.5	0.771	99.8±21.2	0.003
C/C	432	108.1±78.3		62.9±16.1		100.4±21.2	

*P values of analysis of covariance (ANCOVA) adjusting for age, sex, smoking and drinking behaviors.
FTG: fasting triglycerides; HDL-C: HDL cholesterol; FBS: fasting blood sugar.

action between *GCKR* rs780094 *A/A* genotype and low carbohydrate intake (OR for interaction = 1.73; 95% CI 1.06-2.84, P = 0.030) on the risk of dyslipidemia (Table 6). We also investigated all the interactions between *APOA5*, *GCK* and *GCKR* polymorphisms and the selected lifestyle factors on the risk of dysglycemia, which resulted in insignificant interactions. The analyses adjusting for the institutions did not substantially alter the unadjusted ORs, either.

Discussion

In this study, we found significant associations of *APOA5* T-1131C and *APOA5* G553T polymorphisms with the risk of dyslipidemia, serum TG and HDL-C levels, together with the significant associations of *GCK* and *GCKR* polymorphisms with fasting serum glucose levels. In addition, we observed the significant association of *APOA5* T-1131C polymorphism with the risk of dysglycemia.

The associations of *APOA5* T-1131C polymorphism and lipid metabolism disorders have already been reported by several groups [8, 21, 22], and our study confirmed the influence of this *APOA5* T-1131C polymorphism on the risk of dyslipidemia. Meanwhile, few reports have been made about the association between *APOA5* G553T polymorphism and the risk of dyslipidemia to date [9, 10] Our study revealed that the influence of this *APOA5* G553T polymorphism on the genesis of lipid disorder is remarkably strong, even stronger than the *APOA5* T-1131C polymorphism, although the minor allele frequency is rather lower than the *APOA5* T-1131C polymorphism in Japanese. The possible association of *APOA5* T-1131C with the risk of dysglycemia is also in line with the observation in other studies, suggesting the influence of this polymorphism also on glucose metabolism [23, 24]. The possible influence of fat intake on the risk of dyslipidemia through the modulation of insulin sensitivity and other related pathways has been already discussed [25, 26], and the present study results added the novel evidence that genetic factors involved in lipid metabolism also play important roles in this fat intake-induced dyslipidemia.

The associations of *GCK* and *GCKR* polymorphisms with glucose metabolism disorders have been also well described [27, 28], and our study results were in line with these previous findings, underscoring the importance of these associations. We also observed a possible risk reduction of dyslipidemia in those with *GCKR* rs780094 *G/G* genotype or *GCKR* rs1260326 *C/C* gen-

Table 5 Effects of *APOA5* polymorphisms on the risk of dyslipidemia: interactions with dietary intakes and smoking.

	APOA5 T-1131C			*APOA5* G553T		
Genotype	*T/T*	*T/C+C/C*	$P_{interaction}$	*G/G*	*G/T+T/T*	$P_{interaction}$
Calorie intake (kcal/day)						
< standard body weight * 30						
dyslipidemia	58	135	0.969	151	42	0.229
non-dyslipidemia	331	396		658	69	
aOR (95%CI)	1	2.05 (1.45-2.90)		1	2.93 (1.90-4.54)	
≥ standard body weight * 30						
dyslipidemia	74	164		195	43	
non-dyslipidemia	418	454		785	87	
aOR (95%CI)	0.97 (0.67-1.42)	2.01 (1.43-2.82)		1.03 (0.81-1.32)	2.10 (1.38-3.20)	
Fat intake (energy%)						
< 25						
dyslipidemia	110	230	0.183	286	54	0.001
non-dyslipidemia	496	557		948	105	
aOR (95%CI)	1	1.89 (1.46-2.46)		1	1.75 (1.22-2.51)	
≥ 25						
dyslipidemia	22	69		60	31	
non-dyslipidemia	253	293		495	51	
aOR (95%CI)	0.57 (0.35-0.93)	1.59 (1.11-2.28)		0.58 (0.42-0.80)	2.13 (1.26-3.59)	
Carbohydrate intake (energy%)						
< 60						
dyslipidemia	77	208	0.016	223	62	0.073
non-dyslipidemia	534	586		1,016	104	
aOR (95%CI)	1	2.54 (1.89-3.40)		1	2.90 (2.03-4.15)	
≥ 60						
dyslipidemia	55	91		123	23	
non-dyslipidemia	215	264		427	52	
aOR (95%CI)	1.45 (0.98-2.14)	2.02 (1.43-2.86)		1.08 (0.84-1.40)	1.74 (1.03-2.93)	
Alcohol intake (g/day)						
< 23						
dyslipidemia	72	203	0.754	213	62	0.256
non-dyslipidemia	585	679		1,141	123	
aOR (95%CI)	1	2.45 (1.82-3.29)		1	2.82 (2.00-3.99)	
≥ 23						
dyslipidemia	60	96		133	23	
non-dyslipidemia	164	171		302	33	
aOR (95%CI)	2.03 (1.37-3.03)	3.20 (2.23-4.60)		1.63 (1.24-2.13)	2.64 (1.50-4.63)	
Smoking status						
Non-smoker						
dyslipidemia	81	226	0.118	241	66	0.605
non-dyslipidemia	636	745		1,238	143	
aOR (95%CI)	1	2.41 (1.82-3.18)		1	2.42 (1.74-3.37)	
Current smoker						
dyslipidemia	51	73		105	19	
non-dyslipidemia	113	105		205	13	
aOR (95%CI)	2.61 (1.72-3.95)	4.12 (2.79-6.07)		1.93 (1.45-2.57)	5.82 (2.80-12.07)	

aOR: Adjusted Odds Ratio (adjusted for age and sex);
95% CI: 95% confidence interval.

Table 6 Effects of *GCKR* polymorphisms on the risk of dyslipidemia: interactions with dietary intakes.

	GCKR rs780094			*GCKR* rs1260326		
Genotype	*G/A+G/G*	*A/A*	$P_{interaction}$	*T/C+C/C*	*T/T*	$P_{interaction}$
Fat intake (energy%)						
< 25						
dyslipidemia	224	116	0.750	224	116	0.561
non-dyslipidemia	750	303		746	307	
aOR (95%CI)	1	1.26 (0.97-1.64)		1	1.29 (0.99-1.68)	
≥ 25						
dyslipidemia	62	29		61	30	
non-dyslipidemia	404	142		404	142	
aOR (95%CI)	0.72 (0.52-1.00)	1.06 (0.68-1.66)		0.73 (0.53-1.02)	1.03 (0.66-1.62)	
Carbohydrate intake (energy%)						
≥ 60						
dyslipidemia	100	46	0.030	98	48	0.070
non-dyslipidemia	327	152		326	153	
aOR (95%CI)	1	0.92 (0.62-1.38)		1	0.98 (0.66-1.46)	
< 60						
dyslipidemia	186	99		187	98	
non-dyslipidemia	827	293		824	296	
aOR (95%CI)	0.86 (0.65-1.14)	1.38 (0.99-1.92)		0.89 (0.67-1.18)	1.37 (0.98-1.91)	

aOR: Adjusted Odds Ratio (adjusted for age and sex);
95% CI: 95% confidence interval.

otype as shown in Table 2, which seems to be explained by the reduction in serum TG levels observed in Table 4. This is in the similar direction to the previous study results that revealed higher serum TG levels in those with *GCKR* rs780094 *T/T* [29] or *GCKR* rs1260326 *T/T* genotype [5], although the *P*-values were insignificant after adjustment for multiple comparisons.

Our examination of the gene-environment interaction revealed a positive interaction between *APOA5* G553T polymorphism and fat intake, while negative interactions between *APOA5* polymorphisms and carbohydrate intake were also observed. We speculate these results would indicate that if those with *APOA5* G553T high risk genotypes (*APOA5* 553 *G/T* and *T/T*) take higher amount of fat, the risk of dyslipidemia increases much more than in those with the low risk genotype. The possible negative (inverse) interaction between *APOA5* G553T polymorphism and carbohydrate intake observed would be just the reflection of this effect of fat intake. The statistically significant inverse interaction between *APOA5* T-1131C polymorphism and carbohydrate intake would also be explained as the reflection of the theoretically possible interaction between the *APOA5* T-1131C polymorphisms and fat intake as suggested by our study results. The significant interaction observed between *GCKR* rs780094 *A/A* genotype and low carbohydrate intake on the risk of dyslipidemia might also be the reflection of the possible positive interaction between these *GCKR* genotypes and high fat intake although not indicated by the data on Table 6. While a considerable number of studies have demonstrated that higher fat intake is one of the unfavorable risk factors for atherosclerosis or cardiovascular diseases in humans [30, 31] and some studies have demonstrated the influence of *GCKR* and *APOA5* polymorphisms on the postprandial serum TG [32], no previous large population-based observational study seem to have clarified the interaction of these polymorphisms and daily nutrient intakes on the serum TG levels. Our findings would add a novel evidence of genetic predisposition to dyslipidemia induced by high fat intake. These results would possibly provide clues for the establishment of personalized prevention of lipid disorders in the near future.

Consideration of the biological aspects of *APOA5*, *GCK* and *GCKR* polymorphisms is as follows. APOA5 interaction with heparan sulfate proteoglycans (HSPGs) is shown to facilitate apoC-II activation of lipoprotein lipase (LPL), resulting in accelerated triacylglycerol hydrolysis [33]. The *APOA5* T-1131C and G553T

polymorphisms are located in the promoter region and the translated region of *APOA5,* respectively, and thus the *APOA5* T-1131C polymorphism is thought to modulate the expression of *APOA5*, while *APOA5* G553T polymorphism is speculated to affect the function of APOA5 by the substitution of Cys for Gly [10]. As for GCK and GCKR, GCKR normally exists in the nucleus suppressing the GCK function by binding to GCK in the fast, postabsorptive phase, and when a carbohydrate containing meal comes during the ingestive or postprandial phase, the GCKR-GCK interaction is loosened, thus allowing GCK to bind glucose, adopt the catalytically active closed form, and exit from the nucleus to generate glucose-6-phosphate for glycogen synthesis and glycolysis [34]. The *GCKR* rs1260326 and rs780094 polymorphisms are located in the coding region (Leu/Pro at codon 446) and intron 16 of *GCKR* respectively, and these two SNPs are in strong linkage disequilibrium, thus making it difficult to determine which SNP is responsible for the functional change of GCKR at present [5].

Consideration of the technical aspects is as follows. In this study 431 subjects with dyslipidemia / 1,599 subjects without dyslipidemia, and 283 subjects with dysglycemia / 1,747 subjects without dysglycemia were enrolled. The statistical power for 431 cases / 1,599 non-cases is more than 99% when a genotype frequency among the controls is between 20% and 80%, and more than 85% when a genotype frequency among the controls is between 10% and 90% under the same conditions. Moreover, the power for the Wald-test to detect the interaction with the magnitude of effect observed between *APOA5* 553 *G/T+T/T* genotypes and fat intake > 25% of energy on the risk of dyslipidemia (OR = 3.03, standard error = 0.99) resulted in more than 90% (92.2%), based on the power function formula of Wald-test for interaction [35, 36]. Similarly, the power for the interaction between *APOA5* -1131 *T/C+C/C* genotypes and carbohydrate intake > 60% of energy on the risk of dyslipidemia (OR = 0.55, standard error = 0.14) resulted in more than 60% (67.7%), and that for the interaction between *GCKR* rs780094 *A/A* genotype and carbohydrate intake < 60% of energy on the risk of dyslipidemia (OR = 1.73, standard error = 0.44) resulted in more than 50% (58.3%). The statistical power for this sample can be considered strong enough if we examine the association between one exposure (gene or environment) and the outcome (disease). When we consider examining gene-environment interaction, the power for this sample is considered sufficient to detect the interaction between *APOA5* 553 *G/T+T/T* genotypes and fat intake > 25% of energy on the risk of dyslipidemia. Additional studies might be required, however, to confirm the other two interactions observed (those between *APOA5* -1131 *T/C+C/C* genotypes and carbohydrate intake > 60% of energy, and between *GCKR* rs780094 *A/A* genotype and carbohydrate intake < 60% of energy on the risk of dyslipidemia). We adopted the criteria adjusted by Bonferroni's correction for the *p*-values only in the analyses conducted under confirmatory contexts (*i.e.*, in calculating the aORs of *APOA5*, *GCK* and *GCKR* polymorphisms [single SNPs] for dyslipidemia and dysglycemia in Table 2 & Table 3). Considering that there are a number of criticisms suggesting that correction of multiple comparisons by Bonferroni procedures is sometimes too conservative [37, 38], and the wide use of Bonferroni procedures may even be aggravating the tendency of researchers not to present nonsignificant results because presentation of more tests with nonsignificant results may make previously 'significant' results 'nonsignificant' under Bonferroni procedures [39], our judgment may well be justifiable.

In conclusion, our cross-sectional study revealed the essential roles of the polymorphisms of the *APOA5*, *GCK* and *GCKR* in the dysregulations of lipid profiles and blood glucose levels in Japanese, and subsequent combined analyses with lifestyle factors suggested the importance of the gene-environment interaction between the *APOA5* / *GCKR* polymorphisms and dietary intake in the individualized prevention of these lipid metabolism disorders. Further investigations are also expected to confirm these interactions.

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Conflict of interest

The authors declare no conflict of interest.

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