

ORIGINAL

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 $\geq 25\%$ 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*, 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 $\text{FTG} \geq 150$ mg/dL and/or $\text{HDL-C} < 40$ mg/dL, and that for dysglycemia is $\text{FBS} \geq 110$ 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 quit 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 \pm 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 'haplogit' command of STATA adjusted for age and sex based on the EM algorithm [20]. The linkage disequilibrium (LD) between the polymorphisms in two loci (D' and r^2) was estimated by the 'pwnd' 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 dys-

lipidemia 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] $\times 2$ [homozygous minor & heterozygous] $\times 2$ [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 \pm 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, $\chi^2 = 0.893$, $P = 0.345$), 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, $\chi^2 = 0.736$, $P = 0.391$), 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, $\chi^2 < 0.001$, $P = 0.983$), 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, $\chi^2 = 0.268$, $P = 0.605$), 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, $\chi^2 = 0.059$, $P = 0.807$). 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
HbA1c (%)*	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 HbA1c 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 [†]
<i>APOA5</i> 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
<i>APOA5</i> 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
<i>GCK</i> 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
<i>GCKR</i> 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
<i>GCKR</i> 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 [†]
<i>APOA5</i> 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
<i>APOA5</i> 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
<i>GCK</i> 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
<i>GCKR</i> 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
<i>GCKR</i> 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)	<i>P</i> *	HDL-C (mg/dL)	<i>P</i> *	FBS (mg/dL)	<i>P</i> *
<i>APOA5</i> rs662799 (T-1131C)							
<i>T/T</i>	881	98.4±55.3		65.5±16.3		97.9±16.5	
<i>T/C</i>	898	117.1±79.3	<0.001	63.5±16.7	<0.001	99.6±20.3	0.006
<i>C/C</i>	251	152.7±118.2		58.8±15.0		101.4±22.7	
<i>APOA5</i> rs2075291 (G553T)							
<i>G/G</i>	1,789	108.9±71.4		64.3±16.4		99.2±19.4	
<i>G/T</i>	231	140.3±102.4	<0.001	60.7±16.6	<0.001	98.5±17.7	0.777
<i>T/T</i>	10	290.3±207.6		49.2±13.6		94.5±10.4	
<i>GCK</i> rs1799884 (G-30A)							
<i>G/G</i>	1,388	114.9±81.6		63.3±16.2		98.8±20.3	
<i>G/A</i>	581	109.2±71.4	0.410	65.1±17.0	0.132	99.4±16.4	0.285
<i>A/A</i>	61	118.1±60.2		63.1±15.2		101.8±16.1	
<i>GCKR</i> rs780094 (A/G at intron 16)							
<i>A/A</i>	590	119.8±75.1		64.0±16.4		97.2±17.4	
<i>G/A</i>	998	111.7±80.0	0.055	64.0±16.5	0.741	99.4±20.8	0.019
<i>G/G</i>	442	108.6±78.0		63.0±16.1		100.6±17.1	
<i>GCKR</i> rs1260326 (Leu446Pro, T1403C)							
<i>T/T</i>	595	119.2±73.5		63.9±16.6		96.9±16.0	
<i>T/C</i>	1,003	112.2±80.7	0.060	64.1±16.5	0.771	99.8±21.2	0.003
<i>C/C</i>	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.

Genotype	APOA5 T-1131C		<i>P</i> _{interaction}	APOA5 G553T		<i>P</i> _{interaction}
	<i>T/T</i>	<i>T/C+C/C</i>		<i>G/G</i>	<i>G/T+T/T</i>	
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.

Genotype	<i>GCKR</i> rs780094			<i>GCKR</i> rs1260326		
	<i>G/A+G/G</i>	<i>A/A</i>	<i>P_{interaction}</i>	<i>T/C+C/C</i>	<i>T/T</i>	<i>P_{interaction}</i>
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	0.72 (0.52-1.00)	61	30	1.03 (0.66-1.62)
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	0.86 (0.65-1.14)	187	98	1.37 (0.98-1.91)
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 sig-

nificant 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 Tables 2 and 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 non-significant 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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