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Genome-wide association studies have identified the cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (CDKAL1) gene as a novel risk factor for type 2 diabetes mellitus. Application of this genetic marker for prevention of type 2 diabetes and metabolic syndrome (MetS) in healthy populations has not yet been evaluated. The authors examined the effects of a CDKAL1 polymorphism (rs9465871) on metabolic phenotype and of gene-lifestyle (CDKAL1-energy intake) interaction on MetS in a cohort of apparently healthy Japanese men examined in 2003. The CC genotype of the CDKAL1 variant was associated with elevated glycosylated hemoglobin A1c (HbA1c) levels. The prevalence of MetS was 25.6% for CC and 16.3% for TT + CT (odds ratio = 2.18, 95% confidence interval: 1.06, 4.48; \( P = 0.035 \)). When dietary energy intake was accounted for, the variant's effect on HbA1c was observed in the highest energy-intake group (mean: CC, 5.6% (standard deviation, 1.7); TT + CT, 5.0% (standard deviation, 0.5); \( P = 0.025 \)). In addition, the positive association between HbA1c and energy intake was stronger in subjects with the CC genotype than in subjects with TT + CT. These results suggest that the interaction between the CDKAL1 polymorphism and dietary energy intake influences the dysglycemic phenotype leading to MetS, possibly through impaired insulin secretion. The CDKAL1 polymorphism may be a marker for MetS in the Japanese population.

CDKAL1 protein, human; energy intake; hemoglobin A1c protein, human; Japan; metabolic syndrome X

Abbreviations: CDK5, cyclin-dependent kinase 5; CDKAL1, CDK5 regulatory subunit-associated protein 1-like 1; HbA1c, hemoglobin A1c; MetS, metabolic syndrome; PCR, polymerase chain reaction; SD, standard deviation.

Editor's note: An invited commentary on this article appears on page 992, and the authors' response appears on page 998.

Metabolic syndrome (MetS) is characterized by abdominal obesity, hypertension, dyslipidemia, and glucose intolerance (1) and is considered a serious health hazard in developed countries. MetS occurs in up to 22% and 14.7% of US and Japanese adults, respectively (2, 3). In addition, MetS is an independent predictor of the development of type 2 diabetes mellitus and cardiovascular disease (4, 5). The development and progression of MetS is probably affected by a complex interaction of genetic and environmental factors, but the genetic factors that influence MetS remain mostly unknown. However, certain genetic factors affecting MetS may play a role in obesity, inflammation, insulin resistance, and/or glucose and lipoprotein metabolism (6). Our previous studies showed that a gene altered during inflammation, interleukin 6 receptor (IL6R), and a gene altered due to obesity, cell death-inducing DNA fragmentation factor \( \alpha \)-like effector A (CIDEA), are both associated with MetS (7, 8).
Recently, genome-wide association studies identified multiple gene products involved in polygenic diseases, such as type 2 diabetes; cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1 (CDKAL1) was identified by the Wellcome Trust Case-Control Consortium for its association with type 2 diabetes (9). Subsequent studies in different populations have successively corroborated this association, and CDKAL1 is now considered to have a bona fide association with type 2 diabetes in both Western and Japanese populations (10–16). The whole-genome approach is useful for identifying new markers of genetic associations, but determining the effect under the influence of lifestyle factors, such as energy intake, is important. Genome-wide association studies and replication studies have confirmed CDKAL1 as a genetic marker in several European populations, but there have been only a few replication studies in Japanese. Takeuchi et al. (13) reported that CDKAL1 is the best-replicated susceptibility locus and the strongest associated with type 2 diabetes among several susceptibility loci, including KCNQ1 (potassium voltage-gated channel, KQT-like subfamily, member 1), in the Japanese population. Recent studies have shown that type 2 diabetes has a strong positive correlation with total energy intake, which could be a more significant risk factor for type 2 diabetes (17, 18). In addition, the gene-lifestyle (CDKAL1-energy intake) interaction effect on type 2 diabetes and MetS has not yet been examined in the Japanese population. Therefore, we studied the effect of this genetic marker, the CDKAL1 polymorphism, to determine whether it affects the glycemic phenotype, as well as the effect of the gene-lifestyle interaction on MetS in apparently healthy Japanese.

CDKAL1 is expressed in human pancreatic islet cells and shares homology with CDK5 regulatory subunit-associated protein 1, an inhibitor of CDK5 (10). CDK5 has been reported to regulate insulin secretion (19) and maintain β-cell function under glucotoxic conditions (20). Thus, CDKAL1 probably plays a role in the regulation of insulin secretion from pancreatic β cells, even under glucotoxic conditions. To the best of our knowledge, no one has reported an interaction effect of the CDKAL1 polymorphism and lifestyle-related energy intake on type 2 diabetes and MetS, since the pathogenesis of MetS is likely to be affected by gene-lifestyle interactions. We investigated whether CDKAL1 is associated with MetS and whether an interaction effect of this polymorphism and energy intake on MetS is present in healthy Japanese men.

MATERIALS AND METHODS

Study subjects

A total of 313 male Japanese workers from Kanagawa Prefecture, Japan, underwent a health examination in 2003 and were enrolled in this study. Written informed consent was obtained from each participant. Detailed descriptions of the participants are provided elsewhere (7, 8). The mean age and body mass index (weight (kg)/height (m)²) of the participants were 45.7 years (standard deviation (SD), 11.7) and 23.4 (SD, 3.5), respectively. Fifty-eight subjects (18.5%) were diagnosed with MetS, among whom 7 were treated with antidiabetes medication and 37 were treated with antihypertensive medication. The present study was approved by the Ethics Review Committee of the Medical Research Institute of Tokyo Medical and Dental University. MetS was defined according to the criteria of the Japanese Society of Internal Medicine (21) for men as a waist circumference of ≥85 cm and 2 or more of the following: 1) dyslipidemia, serum triglyceride level ≥150 mg/dL, and/or high density lipoprotein cholesterol level <40 mg/dL; 2) high blood pressure (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mm Hg); and 3) impaired fasting glucose concentration (≥110 mg/dL).

Phenotype measurements

Height, weight, systolic blood pressure, and diastolic blood pressure were measured for each participant. All subjects fasted for 12 hours before blood collection. Plasma glucose, serum glycosylated hemoglobin A1c (HbA1c), serum triglyceride, total cholesterol, and high density lipoprotein cholesterol levels were measured. Body mass index was calculated as weight (kg) divided by height squared (m²). Data on the participants’ age, family history of diabetes, and current smoking and alcohol drinking status were obtained using a self-reported questionnaire. Dietary energy intake was calculated from a validated semiquantitative food frequency questionnaire, which was evaluated by a comparison with the 7-day dietary records of 66 subjects (22). The multiple correlation coefficients (R²) for correlation between the 2 methods were 0.217, 0.173, 0.150, and 0.238 for energy, protein, lipids, and carbohydrates, respectively (22). The food frequency questionnaire method has been previously described in detail (7, 8, 23). We classified the subjects into tertile groups based on their energy intake levels in order to obtain an approximate number of subjects for the comparative analysis: low, 2,474.5–6,788.6 kJ/day; middle, 6,790.3–8,465.6 kJ/day; and high, 8,499.9–17,740.0 kJ/day.

DNA genotyping

Peripheral blood was drawn from each subject, and genomic DNA was extracted using a standard method (7, 8). The CDKAL1 polymorphism rs9465871, which is in very high linkage disequilibrium with the rs7756992 polymorphism (D’ = 1.0, r² = 0.91) used in replication studies (12), was selected on the basis of previous genome-wide association studies by the Wellcome Trust Case-Control Consortium (for the association with type 2 diabetes, genotype P value = 3.34 × 10⁻⁷) (9). The analysis of rs9465871 was performed by polymerase chain reaction (PCR), followed by a melting curve analysis using the LightCycler 480 System (Roche Diagnostics, Penzberg, Germany). PCR primers and hybridization probes used were as follows: 5’-CAG TAG AGG TGG AGG AAG-3’ (sense primer), 5’-TCA TCA GAA CAA CCA CCA GT-3’ (antisense primer), 5’-GCA TGT TTC CAG AAG GAG GAG TGA CTC GTG TA-3’ conjugated to fluorescein (sensor probe), and 5’-LC Red 640-GTG TTG CTG AGA AAC TGA GGT AGA T-Phosphate (anchor probe). PCR was performed in a reaction...
mixture containing 0.05 μM sense primer, 0.5 μM antisense primer, 0.2 μM anchor probe, 0.2 μM sensor probe, 10 ng of genomic DNA, and 1 μL of 5X LightCycler 480 genotyping master solution (modified Taq DNA polymerase, reaction buffer, dNTP mix, and 15 mM magnesium chloride) in a total volume of 5 μL. The reaction parameters consisted of a 10-minute initial denaturation at 95°C followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 15 seconds. After PCR amplification, a melting curve analysis was performed by the reaction’s being held at 95°C for 1 minute and at 40°C for 1 minute and then slowly heated to 85°C with a ramp rate of 2°C per second. The melting curve data were collected and classified using the LightCycler genotyping software.

Statistical analysis

The allele frequency was calculated, and the deviation of the genotype distribution from Hardy-Weinberg equilibrium was determined using a chi-square test. Analysis of variance with or without adjustment (age and height) was used to compare the mean values of basic and clinical characteristics and energy intake levels among genotype groups. The odds ratio and 95% confidence interval for the prevalence of MetS between different genotype groups was evaluated using a multiple logistic regression model with or without adjustment for age and height. Furthermore, the correlations between Hba1c level (a continuous variable) and CDKAL1 genotype among the energy intake groups, or the energy intake levels among the genotype groups, were examined by multiple linear regression analyses, which were also adjusted for age, height, or current smoking and alcohol drinking status. Seven subjects were excluded because of antidiabetes treatment. Analysis of covariance was used to examine the gene-environment interaction effects on Hba1c and fasting blood glucose level. SPSS for Windows, version 11.0 (SPSS Inc., Chicago, Illinois), was used for all analyses. A P value less than 0.05 was considered significant. The corrected significance threshold level using the Bonferroni method, based on these 2 interaction tests, was P = 0.05/2 = 0.025. Although we analyzed the gene-environment interaction effect on the blood glucose level, we discuss the effect related to Hba1c rather than to fasting blood glucose.

RESULTS

The frequency of the minor allele, C, was 0.492, which was similar to the frequency in the Japanese general population (0.456; http://www.hapmap.org/). The frequencies of the CC, CT, and TT genotypes of rs9465871 among the 313 Japanese men were 25.2%, 47.9%, and 26.8%, respectively. The genotype distribution did not deviate from Hardy-Weinberg equilibrium (P = 0.46).

Subjects were characterized according to age, height, body weight, body mass index, waist circumference, blood pressure, serum lipid level, fasting plasma glucose concentration, and Hba1c level and were categorized into 3 groups based on their genotype: CC, CT, and TT. There was no significant linear trend for the mean age- and height-adjusted differences in age, body weight, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, serum lipids, fasting plasma glucose, Hba1c, or age-adjusted height among the 3 CDKAL1 genotype groups (data not shown). The adjusted mean Hba1c level in the CC genotype group (5.2% (SD, 1.2)) was higher than that in the CT group (5.0% (SD, 0.85); P = 0.10) or the TT group (4.99% (SD, 0.60)), but the difference was not significant (P = 0.07). Thus, we further classified the participants into 2 groups: TT + CT and CC, which was the homozygous recessive genotype associated with type 2 diabetes (9) and impaired fasting glucose in the previous replication study (24). We found no significant differences in age, height, body weight, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, serum lipid, or fasting plasma glucose levels between the TT + CT and CC genotype groups, even after adjustment for relevant confounding variables (Table 1). In addition, we found no significant differences for the lifestyle factors of dietary energy intake and current smoking and alcohol drinking status when comparing the TT + CT and CC genotype groups (Table 1). However, Hba1c levels were significantly higher in the CC group than in the TT + CT group (P = 0.04). Even after we excluded the 7 subjects with diabetes, carriers of the CC genotype had a significantly higher mean Hba1c level than persons with the TT + CT genotype (P = 0.039, linear regression) after adjustment for age, height, current smoking and drinking status, and energy intake.

The prevalence of MetS was also significantly different between the 2 CDKAL1 genotype groups, with a prevalence of 25.6% in the CC group and 16.3% in the TT + CT group. After adjustment for age and height, the odds ratio for the CC group as compared with the TT + CT group was 2.18 (95% confidence interval: 1.06, 4.48) (P = 0.035).

We examined not only Hba1c but also fasting glucose concentration according to CDKAL1 genotype. We did not find a significant relation between fasting glucose level and the CDKAL1 variants (data not shown).

When analyzing the Hba1c levels of the CC and TT + CT groups after adjusting for age, height, and current smoking and drinking status and after grouping the subjects according to their dietary energy intake, we found the Hba1c level of the CC group with the highest energy intake to be significantly higher than that of the TT + CT group (5.6% (SD, 1.7) vs. 5.0% (SD, 0.5); P = 0.025; Figure 1). No such difference in Hba1c levels was found in the low-energy-intake group (CC vs. TT + CT: 4.9% (SD, 0.7) vs. 5.1% (SD, 1.1); P = 0.99; Figure 1) or the middle-energy-intake group (CC vs. TT + CT: 5.2% (SD, 0.9) vs. 4.9% (SD, 0.4); P = 0.37; Figure 1). Furthermore, the Hba1c level was also significantly different after accounting for the interaction between energy intake as a categorical variable and CDKAL1 genotype (P = 0.028) and after adjusting for age, height, and current smoking and drinking status. The significant interaction effect (P = 0.028) was marginally greater than the conservative, Bonferroni-corrected effect (P = 0.025).

We also examined the correlation between Hba1c levels and energy intake in subjects with the CC and TT + CT genotypes. The TT + CT group did not show significant
positive correlations between HbA1c and energy intake, but the CC group did show a correlation (Figure 2). In the TT + CT group, the regression coefficient was 0.16, and \( P = 0.012 \) for the trend after adjustment for age, height, and smoking and drinking status (Figure 2A). In the CC group, the regression coefficient was 0.25 and \( P = 0.037 \) for the trend (Figure 2B). Furthermore, when we examined the interaction term for \( CDKAL1 \) genotype and energy intake using the multivariate-adjusted general linear model and adjusted for age, height, and current smoking and drinking status, the positive correlation between HbA1c and energy intake was significantly greater in the CC genotype group than in the TT + CT genotype group \( (P < 0.001; \text{Figure 2C}) \). These results indicate that HbA1c levels tend to be higher in subjects with the CC genotype than in those with the TT + CT genotype when energy intake is higher.

**DISCUSSION**

The present study showed that a polymorphism in the type 2 diabetes risk-conferring \( CDKAL1 \) gene is associated with elevated HbA1c levels and an increased prevalence of MetS in apparently healthy Japanese men. The risk allele and genetic models were the same as in the previous study of type 2 diabetes (9).

HbA1c is a minor component of hemoglobin, and the proportion of HbA1c in total hemoglobin (%) is considered to be an integrated measure of blood glucose concentrations for 6–8 weeks prior to HbA1c measurement (25). Therefore, HbA1c is widely accepted as a standard method for determining long-term glycemic control in diabetic patients (26). HbA1c has also been suggested to serve as a predictor of progression to type 2 diabetes in nondiabetic persons (27, 28), as well as for MetS (29). Sung et al. (29) reported that an HbA1c level of 5.5% represents the value with maximum sensitivity and specificity for the diagnosis of MetS in a large-scale cross-sectional study among nondiabetic South Korean adults. In addition, an HbA1c level of 5.2% or less has been recommended in recent guidelines from the Japanese Ministry of Health, Labour, and Welfare (30) to aid in the diagnosis of MetS. Therefore, although the current effect of \( CDKAL1 \) variants on HbA1c levels is within the normal range and is modest (CC vs. TT + CT: 5.2% (SD, 1.2) vs. 5.0% (SD, 0.8)), we think that this difference is an indication of a subtle dysglycemic phenotype detected prior to the elevation in

### Table 1. Characteristics of Subjects According to Cyclin-Dependent Kinase 5 Regulatory Subunit-Associated Protein 1-Like 1 (\( CDKAL1 \)) Genotype, Japan, 2003

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CC (n = 79)</th>
<th>TT + CT (n = 234)</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>%</td>
<td>Mean (SD)</td>
<td>%</td>
</tr>
<tr>
<td>Age, years</td>
<td>45.5 (11.7)</td>
<td>45.8 (11.7)</td>
<td>0.82</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.1 (6.8)</td>
<td>168.9 (6.1)</td>
<td>0.29</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>66.0 (10.3)</td>
<td>66.8 (11.5)</td>
<td>0.55</td>
</tr>
<tr>
<td>Body mass index( ^{b} )</td>
<td>23.4 (3.4)</td>
<td>23.4 (3.6)</td>
<td>0.90</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>83.6 (9.6)</td>
<td>83.9 (9.7)</td>
<td>0.75</td>
</tr>
<tr>
<td>Serum total cholesterol, mg/dL</td>
<td>208.6 (41.6)</td>
<td>206.3 (35.6)</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum triglycerides, mg/dL</td>
<td>137.8 (87.5)</td>
<td>131.6 (81.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum high density lipoprotein cholesterol, mg/dL</td>
<td>52.9 (13.1)</td>
<td>56.2 (14.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dL</td>
<td>101.3 (34.6)</td>
<td>100.4 (33.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>Serum hemoglobin A1c, %</td>
<td>5.2 (1.2)</td>
<td>5.0 (0.8)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>132.2 (18.8)</td>
<td>135.2 (17.5)</td>
<td>0.21</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>79.5 (13.5)</td>
<td>82.4 (12.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>Energy intake, kJ/day</td>
<td>7,700.8 (1,886.1)</td>
<td>7,724.0 (2,064.1)</td>
<td>0.76</td>
</tr>
<tr>
<td>Current smoking</td>
<td>57.5</td>
<td>60.8</td>
<td>0.62</td>
</tr>
<tr>
<td>Current alcohol consumption</td>
<td>69.9</td>
<td>74.5</td>
<td>0.44</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>17.9</td>
<td>14.1</td>
<td>0.46</td>
</tr>
<tr>
<td>Prevalence of metabolic syndrome</td>
<td>25.6</td>
<td>16.3</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

Abbreviations: ANOVA, analysis of variance; SD, standard deviation.

* \( P < 0.05 \).

\( ^{a} \) \( P \) values were derived using an additive model and were determined by unadjusted ANOVA (age), ANOVA adjusted for age (height), ANOVA adjusted for age and height (other continuous traits), or chi-square test (categorized traits).

\( ^{b} \) Weight (kg)/height (m)\(^{2} \).
fasting plasma glucose. In this regard, notably, normal HbA1c is associated with markers of inflammation in patients with coronary artery disease (31).

Our results, obtained from a cohort of male workers, corroborate previous reports in which CDKAL1 genotype was associated with altered insulin responsiveness (10, 14, 15) or the first phase of insulin secretion in normal subjects (32). Moreover, we showed that the CDKAL1 variant rs9465871 affects HbA1c levels in a manner dependent on increased energy intake, implying that the effect of rs9465871 on glycemic control is influenced by nutritional factors. The association of the CDKAL1 variant with MetS poses an intriguing question regarding the mechanism of the development and progression of the disease. To the best of our knowledge, this is the first study to have found an interaction effect of the type 2 diabetes genetic marker CDKAL1 and a lifestyle-related factor, specifically energy intake, on MetS in a healthy Japanese population.

MetS is comprised of 4 typical components: abdominal obesity, hypertension, dyslipidemia, and impaired fasting glucose concentration. Insulin resistance is thought to play a primary role in the pathophysiology of MetS; in the natural course of the disease, insulin resistance is initially counter-balanced by a compensatory increase in insulin secretion to maintain normal glucose tolerance. However, the increase in insulin secretion is eventually unable to offset the severe degree of insulin resistance, and overt type 2 diabetes develops. Our results suggest that β-cell dysfunction might be involved from the early stages of MetS, and it is tempting to speculate that the dysglycemic component of MetS might already be influenced by impaired insulin secretion. We conclude that CDKAL1, which is known to play a role in insulin secretion and type 2 diabetes, interacts with energy intake and confers a risk for the dysglycemic phenotype of MetS.

Our study had the following limitations. First, dietary energy intake was self-reported, derived from answers to a validated semiquantitative food frequency questionnaire, which might have introduced measurement bias. Second, the study sample size within this cohort was not large enough to detect a gene-nutrient interaction effect, because the statistical power was low. We speculate that the subjects’ unique ethnicity may have been the reason why we detected an interaction. In addition, in a previous study, Jakes et al. (33) reported that energy intake was related to
body size, which is correlated with the energy expenditure of physical activity. In our study, we adjusted for body size as a confounding variable; however, there may have been other residual confounding factors not accounted for in our analyses.

The overlap of this genetic risk factor with impaired insulin secretion in patients with type 2 diabetes and MetS suggests a commonality in their etiology. This issue needs to be considered by taking into account ethnic differences, because β-cell function has been shown to be lower in Japanese-American and Chinese-American populations than in African-American and non-Hispanic white populations (34).

Furthermore, Moore et al. (35) reported on the effect of the diabetes gene on lifestyle interventions among non-diabetic high-risk participants in the Diabetes Prevention Program and found no evidence of interactions between CDKAL1 and lifestyle (or metformin). Thus, the CDKAL1 polymorphism may potentially play a role in the initial phase of dysglycemia. Our findings suggest that CDKAL1 may not only be a good genetic marker for the detection of MetS, even in a healthy population, but also may provide descriptive information about lifestyle modification in the prevention of MetS. However, additional studies are warranted for further exploration of the interaction effect, as well as to determine when and how insulin secretion affects the pathogenesis of MetS.

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