Original Article

Effect of the PPARG2 Pro12Ala Polymorphism and Clinical Risk Factors for Diabetes Mellitus on HbA1c in the Japanese General Population

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ABSTRACT

Background: Although the peroxisome proliferator-activated receptor-γ2 (PPARG2) Pro12Ala gene variant is associated with diabetes mellitus, the associations and interactions of this polymorphism and known clinical risk factors with glycated hemoglobin (HbA1c) remain poorly understood. We investigated if carrying the Ala allele was inversely associated with HbA1c level and examined possible interactions.

Methods: This cross-sectional analysis used data collected from 1281 men and 1356 women aged 40 to 69 years who completed the baseline survey of the Japan Multi-Institutional Collaborative Cohort Study. PPARG2 polymorphism was determined by multiplex polymerase chain reaction (PCR)-based Invader assay. Multiple linear regression and ANCOVA were used to control for confounding variables (age, body mass index [BMI], energy intake, alcohol, smoking, physical activity, and family history of diabetes) and examine possible interactions.

Results: After adjustment, the Ala allele was significantly inversely associated with HbA1c level and examined possible interactions. Older age, BMI, and family history of diabetes were associated with higher HbA1c in both sexes. When stratified by PPARG2 genotype, these associations were observed in subjects with the Pro12Pro genotype but not in Ala allele carriers. A significant interaction of genotype and BMI on HbA1c was observed in women. Older age, BMI, and family history of diabetes were significantly associated with high-normal HbA1c (≥5.7% NGSP), whereas PPARG2 polymorphism was not.

Conclusions: Although PPARG2 Pro12Ala polymorphism might attenuate associations between known risk factors and HbA1c level, it had a small effect on high-normal HbA1c, as compared with clinical risk factors, in the general population.

Key words: peroxisome proliferator-activated receptor-γ2; polymorphism; glycated hemoglobin; interaction

INTRODUCTION

The prevalence of type 2 diabetes mellitus (type 2 diabetes) has markedly increased during the last decade. The number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030 if no urgent action is taken.1,2 According to the 2010 National Health and Nutrition Survey, about 17.4% of Japanese men and 9.6% of Japanese
women may have diabetes. Because age is an important risk factor for type 2 diabetes, the number of patients will further increase in Japan, which will result in a serious public health problem.

Type 2 diabetes is a complex of lifestyle-related clinical risk factors and genetic factors. Among clinical risk factors, increased body mass index (BMI), excessive energy intake, physical inactivity, and smoking have been associated with a higher risk of diabetes, while moderate alcohol use has been associated with a lower risk. Among genetic factors, family history has a large effect on predicting the development of type 2 diabetes.

During the last decade, a number of genetic variants have been examined for their association with type 2 diabetes, and a consistent association has been found for peroxisome proliferator-activated receptor-γ2 (PPARG2). A recent meta-analysis reported that the PPARG2 Pro12Ala polymorphism was associated with a reduction in type 2 diabetes risk and that this association did not differ between Asians and whites. However, the interactions between this polymorphism and known clinical risk factors (including family history of diabetes) for type 2 diabetes risk are not well understood.

Glycated hemoglobin (HbA1c) is commonly used to diagnose diabetes and can also be used to identify individuals at higher risk of developing diabetes. In 2010, the American Diabetes Association suggested that prevention strategies should be particularly intensive among people with a high-normal HbA1c level (5.7%–6.4%, National Glycohemoglobin Standardization Program [NGSP] values), because this population has the greatest risk of developing diabetes. Recently, Heianza et al reported that the predictive value of a high-normal HbA1c for diabetes progression was similar to that of impaired fasting glucose alone in the Japanese population. To identify a genetic factor other than family history that is associated with high-normal HbA1c would be useful for implementing early-prevention strategies against diabetes.

The purpose of this study was to examine if carrying the Ala allele of PPARG2 was inversely associated with HbA1c and if this association modified the effects of known clinical risk factors, family history of diabetes, and their interactions. In addition, we investigated whether PPARG2 polymorphism was associated with high-normal HbA1c after adjusting for possible confounders.

METHODS

Study participants

The Japan Multi-Institutional Collaborative Cohort (J-MICC) Study is a large genome cohort followed to confirm and detect gene–environment interactions in lifestyle-related diseases. The details of the cohort have been described elsewhere. Briefly, the J-MICC Study was initiated 2005, and participants aged 35 to 69 years were enrolled voluntarily from 10 areas of Japan. In the present cross-sectional study, we used data from 4519 participants enrolled throughout Japan during 2004–2008. Written informed consent was obtained from all participants, and the study protocol was approved by the ethics committees of Nagoya University School of Medicine and the participating institutions.

Questionnaire and measurements

A self-administered questionnaire including items on alcohol consumption, smoking, dietary habits, current medication, past disease history, and first-degree family history of diabetes was used for data collection. For dietary assessment, a validated food-frequency questionnaire (FFQ) was used, and intakes of energy, fat, protein, carbohydrates, and ethanol were calculated.

Physical activity was assessed in terms of metabolic equivalents (METs) of daily and leisure-time activity. MET values less than 3 were not counted as physical activity. Participants reported the average time per day spent doing physical work (assigned MET intensity: 4.5 METs), walking (3.0 METs), standing (<3.0 METs, not counted as physical activity), and engaged in sedentary activity (<3.0 METs, not counted as physical activity). Response options were as follows (assigned average time per day in parentheses): none (0), less than 1 hour/day (0.5), 1 to less than 3 hours/day (2.0), 3 to less than 5 hours/day (4.0), 5 to less than 7 hours/day (6.0), 7 to less than 9 hours/day (8.0), 9 to less than 11 hours/day (10.0), and 11 or more hours/day (12.0). MET-hours per day (MET·h/day) of daily activity was estimated for heavy physical work and walking. For leisure-time activity, participants were asked about the frequency and average duration of low-intensity exercise (3.4 METs), moderate-intensity exercise (7.0 METs), and high-intensity level exercise (10 METs). The frequency categories (assigned daily average frequencies in parentheses) for leisure-time activity were almost none (0), 1 to 3 times/month (0.1), 1 to 2 times/week (0.2), 3 to 4 times/week (0.5), and 5 to 6 times/week (0.8). The categories for average duration (assigned average hours per activity in parentheses) were less than 30 minutes (0.3), 30 minutes to less than 1 hour (0.8), 1 to less than 2 hours (1.5), 2 to less than 3 hours (2.5), 3 to less than 4 hours (3.5) and 4 or more hours (4.5). MET·h/day of leisure-time activity was estimated by multiplying the reported daily time spent in each activity by the assigned MET intensity. After summing across daily and leisure-time activity, participants were divided into 4 groups by quartile of MET·h/day and stratified by sex.

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. HbA1c (%) was measured in laboratories in each study area, and the results of these measurements were collected. The equation for conversion from HbA1c (Japan Diabetes Society [JDS]) to HbA1c (NGSP) units is officially certified as follows: NGSP(%) = 1.02 × JDS(%) + 0.25%. According to Heianza et al, the sum of the sensitivity and
specificity for identifying individuals with impaired fasting glucose among those with an HbA1c ranging from 5.7% to 6.4% was highest when HbA1c was 5.7%, so we used 5.7% as the HbA1c cut-off to define high-normal HbA1c.

Genotyping details have been described elsewhere. Briefly, 107 single-nucleotide polymorphisms, including the PPARG2 Pro12Ala gene (rs1801282), were genotyped using a multiplex polymerase chain reaction (PCR)-based Invader assay (Third Wave Technologies, Madison, WI, USA) at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN. Genotype distributions were tested for Hardy–Weinberg equilibrium, and the $P$ value (on the exact test) for the $PPARG2$ Pro12Ala gene was 0.80. After genotyping, data from 6 participants who withdrew from follow-up were excluded from further analysis.

Genotyping of polymorphism

Statistical analysis

In the analysis, we excluded 1877 participants who were missing data on PPARG2 polymorphism ($n = 6$) or HbA1c ($n = 1768$), were on type 2 diabetes medication ($n = 193$), or had a dietary energy intake greater than 4000 kcal/day ($n = 2$). Consequently, data from 1280 men and 1356 women aged 40 to 69 years were included in the analysis. Among these participants, some were missing data on alcohol consumption (20 men and 22 women), BMI (1 man), or physical activity (9 men and 14 women).

All analyses were performed with the SAS statistical software package (Ver. 9.1 for Windows; SAS Institute, Cary, NC, USA). A $P$ value of less than 0.05 was considered statistically significant. Intakes of total energy, fat, protein, and carbohydrates were estimated by the SAS software using the information from the FFQ and standard tables of food composition in Japan (Fifth Revised Edition). The SAS software and FFQ were developed by the same researchers. For comparison of participant characteristics by sex we used the $t$ test (for continuous variables) and the $\chi^2$ test (for categorical variables). Crude and adjusted mean HbA1c values and their 95% CIs were evaluated by least-squares general linear regression, and linear trends were assessed by the statistical significance of the regression coefficient of an ordinal variable for the factor under consideration as follows: age category (35–39, 40–49, 50–59, or 60–69 years), BMI quartile, energy intake quartile, fat intake quartile, protein intake quartile, carbohydrate intake quartile, physical activity quartile, alcohol consumption status (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46.0 grams ethanol/day), smoking status (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/day), first-degree family history of diabetes (positive, negative, or unknown), and PPARG2 genotype (Pro12Pro, Pro12Ala, or Ala12Ala). Regarding family history of diabetes, we did not use data from participants with an unknown family history in the tests for trend. To further explore the effects of PPARG2 genotype and clinical risk factors on HbA1c levels we divided each clinical risk factor into 2 groups as follows: age (35–49 or 50–69 years), BMI (<23.6 or ≥23.6 kg/m² for men and <22.4 or ≥22.4 kg/m² for women), energy intake (<1880 or ≥1880 kcal/day for men and <1540 or ≥1540 kcal/day for women), alcohol consumption status (never, former, or current drinker), and family history (positive or negative). PPARG2 genotype was also divided into 2 groups (Pro12Pro or Ala allele carrier). Because of the low frequency or absence of minor homozygous participants within these groups, they were combined with heterozygous participants. Crude and adjusted mean HbA1c values and their 95% CIs and linear trends were computed with respect to genotype and clinical risk factors. The effect of interactions of PPARG2 genotype and covariates on HbA1c were examined with a multiple regression model. The statistical test for an interaction was applied to a product term of a dichotomous PPARG2 genotype and each covariate (ie, age, BMI, energy intake, alcohol consumption, and family history). On the basis of these 5 interaction tests the corrected significance threshold level, using the Bonferroni method, was $P = 0.05/5 = 0.01$. These analyses were stratified by sex because the distributions of clinical risk factors and mean HbA1c levels were significantly different between men and women.

Odds ratios (ORs) and 95% CIs of PPARG2 genotype and the clinical risk factors for high-normal HbA1c ($≥5.7\%$ NGSP) were estimated using logistic regression models adjusted for potential confounders (age, BMI, energy intake, alcohol consumption, smoking, physical activity, and family history of diabetes). In this analysis we excluded 590 participants (332 men and 258 women) whose family history of diabetes was unknown. For further analysis, we excluded subjects who indicated that they had restricted their food intake due to their results on blood tests for glucose or cholesterol.

RESULTS

The genotype frequency was within the Hardy–Weinberg equilibrium (93.6% in Pro12Pro, 6.3% in Pro12Ala, and 0.1% in Ala12Ala for expected values of 93.7%, 6.1%, and 0.1%, respectively). The background characteristics of the participants are summarized in Table 1. As compared with women, men had significantly higher values for HbA1c, BMI, energy intake, carbohydrate/energy intake, prevalence of current smokers, and prevalence of current drinkers. Women had higher fat/energy and protein/energy intakes. Age, physical activity level, and prevalence of a first-degree family history of diabetes were similar between sexes. Tables 2a and 2b show the associations between HbA1c and clinical risk factors according to sex. Among men and women, the adjusted mean HbA1c was higher in older age.
groups and higher BMI categories and among participants with a first-degree family history of diabetes. In men, energy and alcohol intakes were inversely associated with adjusted mean HbA1c. In subsequent analysis that excluded subjects who had restricted their food intake due to their results on blood testing, the significant inverse association between energy and adjusted HbA1c in men disappeared (P for trend = 0.2786), whereas that between alcohol intake and adjusted HbA1c remained significant (P for trend = 0.0026). Carbohydrate intake was positively associated, and fat and alcohol intakes were inversely associated, with crude mean HbA1c in women; however, these associations disappeared after adjustment for possible confounders. No significant associations of protein intake, physical activity, or smoking with adjusted mean HbA1c were observed for either sex.

Table 3 shows associations between HbA1c and PPARG2 genotypes. The adjusted mean HbA1c was lower in female but not male Ala allele carriers. However, there was no significant interaction between sex and PPARG2 allele on HbA1c.

The associations between HbA1c and important clinical risk factors (Tables 2a and 2b) were examined by PPARG2 genotype and sex (Table 4). Positive associations of age, BMI, and family history with HbA1c were seen among participants with the Pro12Pro genotype but not among Ala allele carriers. In addition, a significant interaction between BMI and PPARG2 genotype on HbA1c was observed in women. However, this interaction was not statistically significant after Bonferroni correction. Energy and alcohol intakes were not significantly associated with HbA1c in analysis stratified by genotype or sex.

The effects of PPARG2 genotype and clinical risk factors on the risk of a high-normal HbA1c (≥5.7% NGSP) are shown in Table 5. Older age, higher BMI, and a family history of diabetes were associated with significantly higher ORs for a high-normal HbA1c. Women and Ala allele carriers had lower ORs for a high-normal HbA1c, though these ORs were not
Table 2b. Crude and adjusted means* (%) and 95% CIs of HbA1c (NGSP) by clinical risk factors and parental family history of diabetes in 1356 women

<table>
<thead>
<tr>
<th>Age</th>
<th>Crude mean (95% CI)</th>
<th>Adjusted mean* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35–39</td>
<td>5.21 (5.08–5.33)</td>
<td>5.25 (5.13–5.38)</td>
</tr>
<tr>
<td>40–49</td>
<td>5.30 (5.23–5.36)</td>
<td>5.31 (5.24–5.37)</td>
</tr>
<tr>
<td>50–59</td>
<td>5.49 (5.45–5.53)</td>
<td>5.50 (5.45–5.54)</td>
</tr>
<tr>
<td>60–69</td>
<td>5.55 (5.51–5.58)</td>
<td>5.54 (5.50–5.58)</td>
</tr>
<tr>
<td><strong>P</strong>_for trend &lt; 0.0001</td>
<td></td>
<td><strong>P</strong>_for trend &lt; 0.0001</td>
</tr>
</tbody>
</table>

Body mass index (kg/m²)

| Q1: <86.2 | 5.39 (5.34–5.45) | 5.43 (5.38–5.48) |
| Q2: 86.2 to <92.4 | 5.40 (5.35–5.45) | 5.40 (5.35–5.45) |
| Q3: 92.4 to <98.3 | 5.51 (5.46–5.56) | 5.50 (5.45–5.55) |
| Q4: ≥98.3 | 5.60 (5.55–5.65) | 5.58 (5.53–5.63) |
| **P**_for trend < 0.0001 |                      | **P**_for trend < 0.0001 |

Energy (kcal/day)

| Q1: <1400 | 5.43 (5.38–5.49) | 5.45 (5.39–5.50) |
| Q2: 1400 to <1540 | 5.51 (5.46–5.56) | 5.51 (5.46–5.56) |
| Q3: 1540 to <1670 | 5.44 (5.39–5.49) | 5.44 (5.39–5.49) |
| Q4: ≥1670 | 5.51 (5.46–5.56) | 5.51 (5.46–5.56) |
| **P**_for trend = 0.1957 |                      | **P**_for trend = 0.3222 |

Fat (energy %)

| Q1: <22 | 5.51 (5.46–5.56) | 5.48 (5.42–5.55) |
| Q2: 22 to <45.9 | 5.52 (5.47–5.57) | 5.52 (5.47–5.57) |
| Q3: 45.9 to <69 | 5.45 (5.39–5.50) | 5.45 (5.40–5.50) |
| Q4: ≥69 | 5.43 (5.38–5.48) | 5.46 (5.41–5.52) |
| **P**_for trend = 0.001 |                      | **P**_for trend = 0.3459 |

Protein (energy %)

| Q1: <12 | 5.47 (5.41–5.52) | 5.49 (5.44–5.54) |
| Q2: 12 to <13.2 | 5.47 (5.42–5.52) | 5.47 (5.42–5.52) |
| Q3: 13.2 to <14.3 | 5.48 (5.43–5.54) | 5.48 (5.43–5.53) |
| Q4: ≥14.3 | 5.49 (5.43–5.54) | 5.47 (5.42–5.52) |
| **P**_for trend = 0.5146 |                      | **P**_for trend = 0.7937 |

Carbohydrate (energy %)

| Q1: <53.2 | 5.40 (5.35–5.45) | 5.45 (5.40–5.51) |
| Q2: 53.2 to <56.3 | 5.48 (5.42–5.53) | 5.48 (5.44–5.55) |
| Q3: 56.3 to <59.1 | 5.49 (5.44–5.54) | 5.46 (5.41–5.51) |
| Q4: ≥59.1 | 5.54 (5.49–5.59) | 5.50 (5.45–5.56) |
| **P**_for trend = 0.0002 |                      | **P**_for trend = 0.3479 |

Physical activity level (MET·h/day)

| Q1: ≤22.9 | 5.42 (5.37–5.47) | 5.44 (5.39–5.49) |
| Q2: 23.0 to 45.9 | 5.49 (5.33–5.65) | 5.53 (5.37–5.68) |
| Q3: ≥46.0 | 5.25 (5.02–5.47) | 5.25 (5.03–5.47) |
| **P**_for trend = 0.1421 |                      | **P**_for trend = 0.3659 |

Alcohol

| Never  | 5.50 (5.47–5.53) | 5.49 (5.46–5.52) |
| Former | 5.40 (5.21–5.60) | 5.40 (5.21–5.59) |
| Current 0.1–22.9_g/d | 5.44 (5.39–5.49) | 5.46 (5.41–5.50) |
| 23.0–45.9_g/d | 5.49 (5.33–5.65) | 5.33 (5.17–5.68) |
| 46.0_g/d | 5.25 (5.02–5.47) | 5.25 (5.03–5.47) |
| **P**_for trend = 0.001 |                      | **P**_for trend = 0.1361 |

Smoking

| Never  | 5.49 (5.46–5.51) | 5.48 (5.45–5.51) |
| Former | 5.39 (5.27–5.52) | 5.44 (5.32–5.57) |
| Current 1–19_cigarttes/d | 5.32 (5.19–5.45) | 5.39 (5.26–5.52) |
| 20–39_cigarttes/d | 5.49 (5.32–5.66) | 5.54 (5.37–5.71) |
| ≥40_cigarttes/d | 5.59 (5.41–5.76) | 5.59 (5.41–5.76) |
| **P**_for trend = 0.0760 |                      | **P**_for trend = 0.5833 |

Family history of diabetes

| Positive | 5.60 (5.53–5.66) | 5.61 (5.55–5.67) |
| Negative | 5.45 (5.42–5.48) | 5.45 (5.42–5.48) |
| Unknown | 5.45 (5.39–5.52) | 5.42 (5.36–5.48) |
| **P**_for trend = 0.0001 |                      | **P**_for trend < 0.0001 |

*Adjusted for age (continuous), body mass index (continuous), energy intake (continuous), physical activity level (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/d), and smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/d) and family history of diabetes (positive, negative, or unknown).

**DISCUSSION**

It is assumed that genetic factors modify the effects of known risk factors for diabetes. PPARG2 is a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily, and it regulates various genes involved in lipid and glucose metabolism. PPARG2 deficiency is believed to improve insulin resistance by decreasing muscle/liver triglyceride content and preventing adipocyte hypertrophy. Thus, the effects of lifestyle-related factors on HbA1c can be modified by the Pro12Ala genotype.

Our large cross-sectional study found significant positive associations of HbA1c with age, BMI, and family history of diabetes in men and women with the Pro12Pro genotype but not in Ala allele carriers. This appears to support the hypothesis that Ala allele carriers are less affected by the clinical risk factors of diabetes and that the Ala allele protects against diabetes development. However, the analysis of Ala allele carriers was underpowered due to the small sample size and failed to detect clinical risk among this subpopulation. Similarly, a significant association of this polymorphism with high-normal HbA1c was not detected, also due to the low prevalence of Ala allele carriers. The OR for high-normal HbA1c associated with this polymorphism was lower than those associated with age, BMI, and family history. Thus, the impact of this polymorphism on diabetes development is probably lesser than the effects of known clinical risk factors. Family history can be considered a surrogate for other genetic factors, as well as for family-related clinical risk factors. In addition, family history is regarded as more appropriate than genotype for prediction of individual risk. Lyssenko et al examined if genetic factors were better than established clinical risk factors at predicting progression to diabetes and found that 11 common genetic variants associated with diabetes risk had smaller effects than family history on the ability to predict diabetes development.

The protective effect of the Ala allele was evident in men and women with higher BMIs, and a significant interaction between Pro12Ala genotype and BMI on HbA1c was observed in women. However, this significant interaction disappeared after Bonferroni correction. Several studies found that insulin sensitivity was higher in overweight or obese people with the Ala allele than in those without it.
The few studies investigating potential obesity–genotype interactions found significant interactions. To the best of our knowledge, our study is the first to examine the interaction between Pro12Ala genotype and BMI on HbA1c in an Asian general population, which has a lower proportion of extremely obese persons as compared with white populations. It is plausible that the Ala allele protects against an increase in HbA1c that would normally arise in obese people, because having the allele prevents adipocyte hypertrophy and insulin resistance. We speculate that the lower frequency of Ala allele carriers among Japanese as compared with whites may partially explain why the prevalence of type 2 diabetes in the Japanese population is not dramatically lower than in Western people, despite the lower prevalence of obesity in Japan.

We found that the Ala allele had a significant protective effect against increased HbA1c in women but not in men and that there were no interactions by sex or genotype. Concerning this sex difference, 2 previous studies reported relationships between the Ala allele and insulin resistance by sex. One Italian study found no association between the Pro12Ala allele and type 2 diabetes in the Japanese population.3,26

### Table 3. Crude and adjusted mean HbA1c by PPAR2 gene polymorphism genotype and sex

<table>
<thead>
<tr>
<th>PPAR2 (rs1801282) genotype</th>
<th>n (1280)</th>
<th>(95% CI)</th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>(95% CI)</th>
<th>n (1356)</th>
<th>(95% CI)</th>
<th>Mean&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
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<tbody>
<tr>
<td>Pro12Pro</td>
<td>1194</td>
<td>5.54</td>
<td>(5.50–5.57)</td>
<td>5.54</td>
<td>(5.50–5.57)</td>
<td>1274</td>
<td>5.48</td>
</tr>
<tr>
<td>Pro12Ala</td>
<td>84</td>
<td>5.60</td>
<td>(5.41–5.78)</td>
<td>5.55</td>
<td>(5.42–5.69)</td>
<td>82</td>
<td>5.36</td>
</tr>
<tr>
<td>Ala12Ala</td>
<td>2</td>
<td>5.40</td>
<td>(4.75–6.05)</td>
<td>5.40</td>
<td>(4.52–6.27)</td>
<td>0</td>
<td>5.38</td>
</tr>
</tbody>
</table>

<sup>a</sup>Crude means.  
<sup>b</sup>Adjusted for age (continuous), body mass index (continuous), energy intake (continuous), physical activity (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/d), and smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/d), and family history of diabetes (positive, negative, or unknown).

### Table 4. Adjusted mean (%) HbA1c by age, BMI, and parental family history of diabetes according to PPAR2 genotype in men and women

<table>
<thead>
<tr>
<th>Age</th>
<th>PP (n = 1194)</th>
<th>PA + AA (n = 86)</th>
<th>P for difference</th>
<th>P for interaction</th>
<th>PP (n = 1274)</th>
<th>PA + AA (n = 82)</th>
<th>P for difference</th>
<th>P for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>35–49</td>
<td>5.43 (5.38–5.48)</td>
<td>5.40 (5.17–5.62)</td>
<td>0.7846</td>
<td>0.7254</td>
<td>5.28 (5.24–5.32)</td>
<td>5.24 (5.11–5.34)</td>
<td>0.5152</td>
<td>0.5552</td>
</tr>
<tr>
<td>50–69</td>
<td>5.56 (5.52–5.61)</td>
<td>5.59 (5.43–5.75)</td>
<td>0.5177</td>
<td>0.6663</td>
<td>5.53 (5.50–5.56)</td>
<td>5.41 (4.93–5.19)</td>
<td>0.0811</td>
<td>0.0131</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 + Q2</td>
<td>5.46 (5.41–5.50)</td>
<td>5.50 (5.34–5.67)</td>
<td>0.5870</td>
<td>0.7500</td>
<td>5.40 (5.37–5.43)</td>
<td>5.43 (5.30–5.55)</td>
<td>0.6232</td>
<td>0.0286</td>
</tr>
<tr>
<td>Q3 + Q4</td>
<td>5.62 (5.56–5.68)</td>
<td>5.60 (5.38–5.82)</td>
<td>0.8779</td>
<td>0.2842</td>
<td>5.57 (5.53–5.61)</td>
<td>5.37 (5.20–5.54)</td>
<td>0.0222</td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 + Q2</td>
<td>5.56 (5.50–5.62)</td>
<td>5.70 (5.49–5.92)</td>
<td>0.2041</td>
<td>0.0673</td>
<td>5.48 (5.45–5.52)</td>
<td>5.34 (5.19–5.50)</td>
<td>0.0764</td>
<td>0.3936</td>
</tr>
<tr>
<td>Q3 + Q4</td>
<td>5.52 (5.48–5.56)</td>
<td>5.39 (5.23–5.55)</td>
<td>0.1407</td>
<td>0.4847</td>
<td>5.48 (5.44–5.52)</td>
<td>5.43 (5.28–5.57)</td>
<td>0.0463</td>
<td>0.4948</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Never + Former</td>
<td>5.60 (5.52–5.67)</td>
<td>5.66 (5.38–5.95)</td>
<td>0.6557</td>
<td>0.7657</td>
<td>5.50 (5.47–5.53)</td>
<td>5.44 (4.96–5.22)</td>
<td>0.3807</td>
<td>0.3695</td>
</tr>
<tr>
<td>Current</td>
<td>5.52 (5.48–5.56)</td>
<td>5.52 (5.36–5.67)</td>
<td>0.9605</td>
<td>0.3742</td>
<td>5.44 (5.40–5.49)</td>
<td>5.32 (5.15–5.49)</td>
<td>0.1774</td>
<td></td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5.73 (5.64–5.81)</td>
<td>5.50 (5.13–5.87)</td>
<td>0.2290</td>
<td>0.5604</td>
<td>5.60 (5.50–5.69)</td>
<td>5.59 (5.02–6.15)</td>
<td>0.9651</td>
<td>0.7307</td>
</tr>
<tr>
<td>Negative</td>
<td>5.51 (5.46–5.55)</td>
<td>5.45 (5.29–5.62)</td>
<td>0.5411</td>
<td>0.5983</td>
<td>5.46 (5.43–5.49)</td>
<td>5.37 (5.26–5.47)</td>
<td>0.0983</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5.50 (5.42–5.58)</td>
<td>5.89 (5.59–6.18)</td>
<td>0.0133</td>
<td>0.3151</td>
<td>5.46 (5.40–5.52)</td>
<td>5.35 (5.14–5.56)</td>
<td>0.3151</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for age (continuous), body mass index (BMI: continuous), energy intake (continuous), physical activity level (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/d), and smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/d), and family history of diabetes (positive, negative, or unknown).

<sup>b</sup>P<sub>diff</sub> and P<sub>interaction</sub> were compared using the adjusted means of individuals with and without a family history of diabetes.
that cause insulin resistance.\textsuperscript{19} Hormonal factors potentially modulate this sex-specific association. \textit{PPARG2} activation has been reported to inhibit aromatase, a key enzyme in the conversion of androgens to estrogens.\textsuperscript{28} In addition, the possibility of sex-specific lifestyle effects on HbA1c should be considered.\textsuperscript{29} In men, significantly higher levels of BMI, energy intake, and cigarette smoking might have greater effects than genotype on HbA1c.

In this study, we observed a favorable effect of alcohol intake on HbA1c in men but not in women. This favorable effect of alcohol intake against high-normal HbA1c was not clear after adjustment for possible confounding factors. A meta-analysis of prospective observational studies noted that moderate alcohol consumption lowered the risk of type 2 diabetes;\textsuperscript{30} however, results from Japanese studies have been inconsistent.\textsuperscript{31} Follow-up of our study is needed to provide further information on the Japanese population.

Our study has several methodological issues that warrant discussion. First, we used HbA1c instead of fasting glucose and insulin to evaluate diabetes risk. However, HbA1c is more commonly used to diagnose diabetes and can be used to identify individuals at higher risk of developing diabetes.\textsuperscript{8} It has been reported that high-normal HbA1c is a strong predictor of type 2 diabetes in the Japanese population.\textsuperscript{6,23} We were aware that excluding 1768 participants with missing HbA1c data might cause selection bias. However, we found that the proportions of \textit{PPARG2} genotypes among the excluded data were not different from those in the analyzed

\begin{table}
\centering
\caption{Odds ratios (ORs) and 95\% CIs for high HbA1c (≥5.7 NGSP) according to genotype and clinical risk factors among 2046 men and women}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
 & \multicolumn{2}{c|}{Model 1} & \multicolumn{2}{c|}{Model 2} & \multicolumn{2}{c|}{Model 3} \\
 & \multicolumn{1}{c|}{n} & \multicolumn{1}{c|}{High HbA1c} & \multicolumn{1}{c|}{OR} & \multicolumn{1}{c|}{95\% CI} & \multicolumn{1}{c|}{OR} & \multicolumn{1}{c|}{95\% CI} \\
\hline
Sex & \multicolumn{3}{c|}{Men} & \multicolumn{2}{c|}{Women} & \\
\hline
 & 231 & 0.88 & (0.72–1.08) & 0.92 & (0.69–1.25) & 1.05 & (0.74–1.49) \\
Model 1 & 948 & 1.00 & (reference) & 1.00 & (reference) & 1.00 & (reference) \\
Model 2 & 1098 & 1.00 & (reference) & 1.00 & (reference) & 1.00 & (reference) \\
Model 3 & \multicolumn{2}{c|}{PPARG Pro12Ala (C/G) (rs1801282)} & \multicolumn{2}{c|}{PA + AA} & \multicolumn{2}{c|}{PA} & \multicolumn{2}{c|}{PP} \\
\hline
P & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} \\
\hline
Age & \multicolumn{2}{c|}{35–39} & \multicolumn{2}{c|}{40–49} & \multicolumn{2}{c|}{50–59} & \multicolumn{2}{c|}{60–69} \\
\hline
Q1 (lowest) & 523 & 1.00 & (reference) & 1.00 & (reference) & 1.00 & (reference) & 1.00 & (reference) \\
Q2 & 515 & 1.47 & (1.06–2.05) & 1.34 & (0.95–1.90) & 1.24 & (0.84–1.83) \\
Q3 & 513 & 2.28 & (1.66–3.13) & 2.04 & (1.46–2.85) & 1.88 & (1.28–2.75) \\
Q4 (highest) & 494 & 3.20 & (2.34–4.34) & 2.87 & (2.07–3.98) & 2.56 & (1.75–3.75) \\
\hline
BMI & \multicolumn{2}{c|}{Q1 (lowest)} & \multicolumn{2}{c|}{Q2} & \multicolumn{2}{c|}{Q3} & \multicolumn{2}{c|}{Q4 (highest)} \\
\hline
P & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} \\
\hline
Energy (kcal/day) & \multicolumn{2}{c|}{Q1 (lowest)} & \multicolumn{2}{c|}{Q2} & \multicolumn{2}{c|}{Q3} & \multicolumn{2}{c|}{Q4 (highest)} \\
\hline
P & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} \\
\hline
Alcohol & \multicolumn{2}{c|}{Never} & \multicolumn{2}{c|}{Former} & \multicolumn{2}{c|}{Current 0.1–22.9 g/d} & \multicolumn{2}{c|}{Current 23.0–45.9 g/d} & \multicolumn{2}{c|}{Current 46.0+ g/d} \\
\hline
P & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} \\
\hline
Family history of diabetes & \multicolumn{2}{c|}{Negative} & \multicolumn{2}{c|}{Positive} & \multicolumn{2}{c|}{Negative} & \multicolumn{2}{c|}{Positive} & \multicolumn{2}{c|}{Negative} & \multicolumn{2}{c|}{Positive} \\
\hline
P & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} & \multicolumn{2}{c|}{<0.0001} \\
\hline
\multicolumn{2}{c}{Model 1: Crude OR.} & \multicolumn{2}{c}{Model 2: Adjusted for PPARG2 genotype (PP or PA + AA), age (continuous), body mass index (BMI; continuous), energy intake (continuous), physical activity (continuous), ethanol intake (never, former, or current drinker consuming 0.1–22.9, 23.0–45.9, or ≥46 g ethanol/d), and smoking (never, former, or current smoker of 1–19, 20–39, or ≥40 cigarettes/d), and family history of diabetes (positive or negative).} & \multicolumn{2}{c}{Model 3: Further excluded subjects who answered that they had restricted food intake due to the results of blood testing for glucose and cholesterol. Adjusted for same variables in Model 2.} & \multicolumn{2}{c}{\textsuperscript{a}Alcohol intake data were missing for 31 subjects.}}
\end{table}
PPARG2 Pro12Ala Polymorphism and HbA1c in Japanese

data. A second limitation of this research is that we did not standardize HbA1c measurements among the laboratories in this study. This is a possible cause for the neutral results regarding the association between HbA1c and PPARG2 genotype. Third, the cross-sectional nature of our study limits our ability to determine causation, even though we excluded participants who were receiving medication for type 2 diabetes. Fourth, there may be intrinsic information bias in our assessments of lifestyle-related factors, dietary factors, and family history. If present, however, any misclassification would be nondifferential with respect to PPARG2 genotype and would likely underestimate the true associations. Finally, residual confounding by known and unknown risk factors may be present, although we adjusted for potential confounding factors in multivariate analysis.

In conclusion, the PPARG2 Pro12Ala polymorphism might modify the risk factors of diabetes. The impact of this allele in the Japanese population appears to be lower than the effects of age, BMI, and family history. These findings highlight the importance of known risk factors, versus genetic polymorphism, in common diseases.

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Conflicts of interest: None declared.

ONLINE ONLY MATERIALS

The Japanese-language abstract for articles can be accessed by clicking on the tab labeled Supplementary materials at the journal website http://dx.doi.org/10.2188/jea.JE20120078.

REFERENCES


