Association of Lifestyle Factors, Polymorphisms in Adiponectin, Perilipin and Hormone Sensitive Lipase, and Clinical Markers in Japanese Males

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Summary According to recent genome-wide association studies, a number of single nucleotide polymorphisms is reported to be associated with diseases or several clinical markers. Among them, adiponectin (ADIPOQ) and perilipin (PLIN) polymorphisms are major factors of obesity. However, the association between lifestyle factor, these polymorphisms and obesity-related clinical markers in Japanese is not well researched. Therefore, the aim of present study is to investigate the association between lifestyle factor, polymorphisms of lipid metabolic genes, and clinical markers in 148 middle-aged Japanese males. The study revealed that ADIPOQ 45 T>G and ADIPOQ 276 G>T genotypes were significantly associated with triglyceride, total cholesterol, hemoglobin A1c (HbA1c) in blood and body mass index (BMI). PLIN4 11 482 G>A and hormone sensitive lipase (LIPE)-60 C>G genotypes were respectively associated with BMI and serum triglyceride. Not only genetic factors but also lifestyle factors influence several clinical markers. The BMI of subjects who like sweets and have the GG allele in ADIPOQ 276 G>T was higher than that of subjects who don’t like sweets. The habit of eating fruits and fish affected low-density lipoprotein-cholesterol of the GT allele and HbA1c of the TT allele in ADIPOQ 276 G>T. Those findings indicate improvement and conservation of lifestyle depending on genetic predisposition in ADIPOQ, PLIN and LIPE should be encouraged.

Key Words genetic association, lifestyle, obesity, SNP

More than 1.1 billion people worldwide are overweight or obese. Obesity, defined as increased adipose tissue mass, is a major risk factor for metabolic disorders such as diabetes mellitus, hypertension, and atherogenic diseases (1, 2). These diseases are influenced by gender, ethnic distinction, genetic factors and daily lifestyle factors such as exercise, habitual smoking and food consumption. Therefore, we are driven by necessity to explore the cause of obesity in consideration of hereditary predisposition and circumstantial conditions (3, 4).

The effect of genetic factors on obesity is being investigated and a great deal of knowledge is accumulating. Menzaghi et al. reported ADIPOQ 45 T>G and ADIPOQ 276 G>T haplotypes were associated with obesity along the east coast of Italy (5). ADIPOQ 276 G>T was also associated with obesity in a study performed in southern Taiwan (6). Although hundreds of candidate genes involved with obesity have been identified now, it is not conclusively clear what gene is responsible for obesity. Furthermore, the influence of genetic type and daily lifestyle factors has not been investigated well (7). Under those circumstances, Qi et al. reported that the genetic predisposition such as hematopoietically expressed homeobox (HHEX: rs1111875) and peroxisome proliferator-activated receptor gamma (PPARG: rs1801282) may synergistically interact with a Western dietary pattern in determining diabetes risk in men (8). Huang et al. suggested that subjects with the genotypes GT and TT at ADIPOQ 276 G>T (rs1501299) had a greater adiponectin-related response to exercise training than those with the GG genotype (9).

In Japan, the rate of obesity also has been increasing dramatically. Obesity mostly occurs in middle-aged men 40–59 y old, which results in the short lifespan of the
Japanese male. The Japanese criterion for obesity (not obesity disease) is a body mass index (BMI) of more than 25 kg/m² (10). Those with a BMI over 25 kg/m² accounts for over 30% of the population of Japanese men aged 40–59 (11). Recent epidemiological studies have revealed that the incidence of obesity-related diseases such as diabetes mellitus, hypertension, and hyperlipidemia is significantly greater among Japanese who exceed BMI 25 kg/m² (12). Additionally, as BMI increases, the percentages of hypertension (diastolic pressure ≥140 mmHg, systolic pressure ≥90 mmHg), hypercholesteremia (serum total cholesterol 240 mg/dL) and high level of serum triglyceride (≥150 mg/dL) cases increase severally (13). Regardless of these circumstances, the study among these single nucleotide polymorphisms (SNPs), obesity-related clinical characteristics and lifestyle factors in Japanese is not well researched. It’s partly because the procedures for clinical study of the human genome face difficulties with the Ethical Review Board.

In this study, we tested adiponectin (ADIPOQ), perilipin (PLIN) and hormone sensitive lipase (LIPE) genes because these genes have been shown to be major players in obesity. Among those genes, the following SNPs were selected: ADIPOQ 45 T>G (rs2241766), ADIPOQ 276 G>T (rs1501299), PLIN1 6209 G>A (rs2289487), PLIN4 11482 G>A (rs894160), PLIN (rs8179043) and LIPE −60 C>G (rs34845087). These genes have been previously reported as causal genes of obesity in some areas (14).

The adiponectin encoded by ADIPOQ is an adipose-specific plasma protein (15, 16). The low level of adiponectin in serum has a close relation with metabolic syndrome symptoms such as insulin resistance and diabetes mellitus (17–19). It has been reported that 40% of Japanese have a hereditary predisposition toward lower adiponectin levels (20).

On the other, the protein encoded by PLIN covers the lipid droplet surface and modulates the turnover of stored fat protecting from LIPE digestion. The PLIN4 polymorphism exists in the sixth intron of the coding sequence and is associated with obesity in women, but not men among Caucasians (21). PLIN1 allelic difference was associated with a lower postprandial response (22).

In addition, the protein encoded by LIPE is expressed in adipose tissue and hydrolyzes stored triglycerides to free fatty acids. The LIPE polymorphism exists 60 bp upstream from the transcription start site, and an association between the G allele and a lower percentage of body fat (% FAT) was reported in Caucasian women, as well as a higher BMI in African-American women (23). However, the association between allele types and obesity is controversial (24).

As mentioned, obesity is a risk factor for a variety of disorders. Thus, in Japanese, the study of genetic factors is important. However, there are only a few published reports. Furthermore, understandings of the effect of lifestyle factors associated with allele types and obesity-related clinical conditions have never been tested before. The genetic factor is not changeable, but the lifestyle can be changed so it is important to discover the effect of lifestyle on the obesity-related clinical data to improve Japanese male health. Therefore, we focused on the association between these SNPs, clinical markers and lifestyle factors in middle-aged Japanese men. We dealt with adult male subjects aged 40 to 60 y old, because the incidence of obesity is increasing in middle-age. If we have some suggestions about lifestyle factors resulting from this study, they may increase the longevity of Japanese men.

**MATERIALS AND METHODS**

**Subjects.** The subjects consisted of 148 Japanese men, 40 to 60 y old, who consulted a physician for their regular health checkup at Fukuoka Institute of Occupational Health (FIOH). Informed consent was obtained from all participants of this study after they had received a written explanation of the study. From these initial subjects, we selected the 74 of the subjects whose waist circumference was more than 85 cm, and the 74 of the subjects whose waist circumference was less than 85 cm at random (in Japanese men, cutoff values of a waist circumference for obesity ≥85 cm have been adopted) (25). Those subjects were typical middle-age Japanese males, as described in “Discussion”.

The research followed all applicable institutional and governmental regulations concerning the ethical use of human volunteers. This research was approved by the Human Genome Research Ethical Review Board of the Institute of Life and Environmental Science for Human Life, Ochanomizu University.

The clinical markers, which are age, height, weight, waist circumference, serum triglyceride, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), blood glucose level, hemoglobin A1c (HbA1c), total protein, and albumin, were measured at FIOH. BMI was calculated from the measurements of height and weight. Serum low-density lipoprotein-cholesterol (LDL-C) was calculated from the values of triglyceride, TC, and HDL-C. For accuracy, fasting blood was used for the measurement of all clinical markers.

**Biochemical measurements.** Fasting blood samples were drawn into tubes for biochemical assays at FIOH. Serum was then obtained by centrifugation at 3,000 rpm and 4°C. Blood markers except for HbA1c were measured on the clinical biochemistry analyzer BioMajesty BM-2250 (JEOL Ltd., Tokyo, Japan). The following agents or kit were used for several clinical measurements: Determiner L TG (Kyowa Medex Co., Ltd., Tokyo, Japan) for triglyceride, Determiner L TC (Kyowa Medex Co., Ltd.) for TC, Cholestest N HDL (Sekisui Chemical Co., Ltd., Tokyo, Japan) for HDL-C. Shikarikid GLU (Kanto Chemical Co., Inc., Tokyo, Japan) for enzymatic measurement of blood glucose, Ekdia XI “Eiken” TP by the biuret method (Eiken Chemical Co., Ltd., Tokyo, Japan) for total protein measurement, and Ekdia XI “Eiken” ALB-BCG by the BCG method (Eiken Chemical Co., Ltd.) for albumin measurement. HbA1c was measured on the automatic glycohemoglobin analyzer HLC-723G8.
DNA extraction and SNP genotyping. Blood samples were collected at FIOH with a Na-EDTA tube, and were immediately cooled and stored at −80°C. Genotyping was carried out at the Institute of Life and Environmental Science for Human Life. DNA was extracted by standard methods using a DNA extraction kit for blood samples (Takara, Kyoto, Japan) according to the manufacturer's instructions. The purified DNA was diluted with TE buffer to 10 ng/μL and stored at −20°C. The SNPs of ADIPOQ (rs2241767) (assay ID C_26426077_10), ADIPOQ (rs1501299) (assay ID C_7497299_10), PLIN4 (assay ID C_8722593_10), PLIN1 (assay ID C_15881785_20), PLIN (rs8179043) (assay ID C_26774469_10) and LIPE (rs1501299) (assay ID C_11482 G>A) were genotyped using TaqMan SNP allelic discrimination by means of an ABI 7300 System (Applied Biosystems, Foster City, CA). Duplicated genotyping was matched 100%.

Definition of obesity and statistical analysis. BMI was used to define obesity according to the Japanese Society for the Study of Obesity. Having a BMI greater than 25 was considered obese for Japanese men. Clinical data are shown as means±SD. Data were analyzed by one-way ANOVA, and the differences among groups were tested by Fisher’s PLSD test using StatView 5.0 software (SAS Institute Inc., Cary, NC). Significance was considered at p<0.05. Chi-square tests were conducted to examine whether the genotype frequencies were in Hardy–Weinberg equilibrium at p<0.05. Analysis of genotype differences was performed by one-way ANOVA and Fisher’s PLSD test at p<0.05. StatView 5.0 software was used to test for distributions of allelic frequencies of SNPs and SNP haplotype.

Questionnaire. Subjects filled in simple questionnaire during the physical test. Questions used for the study were about lifestyle behavior (smoking, exercise, and food consumption habits concerning alcohol, vegetables, fruits, fish and sweets). Answers were given on Likert scales of 3–5 steps. Tobacco and alcohol consumption were scaled by 1: never; 2: not now; 3: low (a glass of alcohol per day/a cigarette per day); 4: moderate (two glasses of alcohol per day/one to 19 cigarettes per day); 5: heavy (three glasses of alcohol per day/ more than 20 cigarettes per day). One glass of alcohol is comparable to “sake 160 mL,” “beer 633 mL,” “tobacco 60 mL,” “wine 190 mL,” “one third cup of distilled spirits, around 60 mL.” Answer 1 and 2 were categorized “No” and, 3, 4 and 5 were “Yes.” Habitual exercise was scaled by 1: do never; 2: light; 3: hard. Food consumption habit was scaled by 1: like it very much; 2: like it slightly; 3: do not like it; 4: do not eat it. Answer 1 and 2 were categorized “Yes” and 3 and 4 were “No”.

RESULTS

Clinical data and genotype distribution of study subjects

Table 1 presents clinical data for the study subjects.

The average age of all subjects was 48.66 ± 5.40 years old. The mean values of BMI and waist circumference were 24.02 ± 5.64 kg/m² and 84.11 ± 10.9 cm, respectively. The mean values of serum triglyceride, TC, HDL-C, and LDL-C were 166.5 ± 39.8 mg/dL, 127.4 ± 34.7 mg/dL, and 53.89 ± 15.1 mg/dL, respectively. The mean values of blood glucose and HbA1c were 104.7 ± 24.5 mg/dL and 5.40 ± 0.81%, respectively. The mean values of serum total protein and albumin were 7.23 ± 0.42 g/dL and 4.57 ± 0.23 g/dL, respectively.

All clinical data for our subjects was similar to those for most Japanese males. Allelic frequencies were as follows: ADIPOQ 45 T>G G=0.240; ADIPOQ 276 G>T T=0.335; PLIN1 6209 G>A A=0.375; PLIN4 11482 G>A A=0.294; PLIN (rs8179043) A=0.612; and LIPE −60 C>G G=0.304. The frequencies were fitted to Hardy–Weinberg equilibrium (Table 2).

The association between genotype and clinical data

The SNP of ADIPOQ 45 T>G was significantly associated with triglyceride, and might have a probability of association with total cholesterol and HbA1c (Table 3). Triglyceride and total cholesterol of the GG genotype were significantly higher than those of the TT genotype. HbA1c of the GG genotype was also significantly higher than that of the GT and TT genotypes. The SNP of ADIPOQ 276 G>T might be associated with BMI and triglyceride (Table 4). BMI of the GG genotype was significantly lower than that of the GT genotype. On the other hand, triglyceride of the GG genotype was higher than that of the TT genotype.

The SNP of PLIN4 was associated with the obesity index of BMI, weight and waist circumference (Table 5). The subjects with the AA genotype demonstrated significantly higher BMI than that for the other genotypes. The weight and waist circumference of subjects with the AA genotype were also higher than those in subjects with the GG genotype. On the other hand, the SNPs of PLIN1 and PLIN (rs8179043) did not show any association with the clinical data.
Qi et al. (26) reported that polymorphism of PLIN was associated with an increased risk of type 2 diabetes in non-obese women; therefore, we analyzed the association between the PLIN4 genotype and other clinical data involved in diabetes in the obese group (BMI ≥ 25) and in the non-obese group (BMI < 25) independently. However, no significant difference was observed in either group.

The relationship between the genotypes of LIPE and the clinical data is described in Table 6. The serum triglyceride level associated with the CG genotype in the LIPE - 60 C>G polymorphism was significantly lower
Table 4. Relation between ADIPOQ 276 G>T genetic polymorphism and clinical data.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>GG (n=63–67)</th>
<th>GT (n=62–63)</th>
<th>TT (n=18)</th>
<th>p (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>48.0±5.38</td>
<td>49.15±5.08</td>
<td>49.00±6.58</td>
<td>0.512</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.1±5.46</td>
<td>169.0±5.23</td>
<td>168.9±5.48</td>
<td>0.991</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.7±9.55</td>
<td>70.45±8.63</td>
<td>66.30±9.30</td>
<td>0.131</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.6±1.80a</td>
<td>24.65±2.66b</td>
<td>23.32±3.15b</td>
<td>0.056</td>
</tr>
<tr>
<td>Waist circ (cm)</td>
<td>83.38±5.78</td>
<td>85.15±5.98</td>
<td>83.06±6.17</td>
<td>0.187</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>187.5±137.4a</td>
<td>146.8±79.2b</td>
<td>157.4±82.3b</td>
<td>0.104</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>219.7±14.7</td>
<td>208.3±38.9</td>
<td>217.3±41.9</td>
<td>0.214</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>53.2±12.53</td>
<td>54.33±12.07</td>
<td>54.8±10.54</td>
<td>0.821</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>129.1±33.3</td>
<td>124.6±35.3</td>
<td>131.1±38.6</td>
<td>0.687</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>106.8±30.2</td>
<td>103.6±17.5</td>
<td>100.8±22.0</td>
<td>0.585</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.13±0.943</td>
<td>5.324±0.670</td>
<td>5.310±0.726</td>
<td>0.359</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.210±0.350</td>
<td>7.271±0.510</td>
<td>7.206±0.300</td>
<td>0.676</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.561±0.247</td>
<td>4.595±0.237</td>
<td>4.589±0.208</td>
<td>0.706</td>
</tr>
</tbody>
</table>

All values are means±SD. a,b Different letters indicate significant differences at p<0.05 among groups by Fisher’s protected least significant difference method.

Table 5. Relation between PLIN4 11482 G>A genetic polymorphism and clinical data.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>GG (n=69–73)</th>
<th>GA (n=61–63)</th>
<th>AA (n=12)</th>
<th>p (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>48.38±5.44</td>
<td>49.30±5.53</td>
<td>46.9±4.14</td>
<td>0.314</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.8±5.67</td>
<td>169.2±5.15</td>
<td>169.5±3.20</td>
<td>0.878</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.8±8.84a</td>
<td>68.63±9.16a</td>
<td>74.75±10.03b</td>
<td>0.054</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.78±2.73a</td>
<td>23.91±2.75a</td>
<td>26.01±3.15b</td>
<td>0.036*</td>
</tr>
<tr>
<td>Waist circ (cm)</td>
<td>83.42±4.92a</td>
<td>84.25±6.77ab</td>
<td>87.42±6.17b</td>
<td>0.096</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>163.8±118.8</td>
<td>170.0±106.1</td>
<td>164.9±90.5</td>
<td>0.949</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>215.2±34.0</td>
<td>215.1±33.4</td>
<td>208.3±44.8</td>
<td>0.832</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>54.0±12.34</td>
<td>53.67±12.24</td>
<td>54.33±9.88</td>
<td>0.978</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>128.4±36.7</td>
<td>127.5±31.9</td>
<td>121.2±38.2</td>
<td>0.780</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>103.4±23.4</td>
<td>105.9±26.7</td>
<td>106.8±19.9</td>
<td>0.792</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.38±0.791</td>
<td>5.43±0.876</td>
<td>5.40±0.623</td>
<td>0.946</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.192±0.493</td>
<td>7.268±0.344</td>
<td>7.333±0.253</td>
<td>0.404</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.574±0.225</td>
<td>4.575±0.261</td>
<td>4.633±0.178</td>
<td>0.713</td>
</tr>
</tbody>
</table>

All values are means±SD. a,b Different letters indicate significant differences at p<0.05 among groups by Fisher’s protected least significant difference method. Asterisk (*) indicates significant difference by one-way ANOVA.

than that associated with the CC genotype. The HDL-C concentration in subjects with the CG genotype was higher than that of subjects with the CC genotype, but not significantly. We also analyzed this genotype effect in the obese and non-obese groups independently, but observed no significant relationship.

The association between genotype, clinical data and lifestyle factors in the questionnaire

Several clinical markers in some genotypes were influenced by daily lifestyle factors. Average BMI of the GG allele in ADIPOQ 276 G>T was lower than that of the GT allele (Table 4), and in the GG allele, the average BMI of the subjects who don’t like sweets (21.98) was significantly lower than that of those who like sweets (24.36) (Fig. 1A).

In the preference for fruits, the average LDL-C has been affected in the GT allele of ADIPOQ 276 G>T (Fig. 1B). In the GT allele, the subjects who like fruits (129.7) had higher LDL-C than those who don’t like fruits (103.4). Average HbA1c of the TT allele in ADIPOQ 276 G>T was influenced by the habit of eating fish. In the TT allele, average HbA1c of subjects who like fish (5.120) was lower than that of subjects who don’t like fish (6.267) (Fig. 1C).

Average total cholesterol in ADIPOQ 45 T>G was 239.6 in the GG allele, and 209.2 in the TT allele, while the GT allele was between these values at 219.5. Exercise habits influenced total cholesterol value for the GT allele (Fig. 1D); the average total cholesterol of the persons who do not exercise (228.5) was significantly higher than that of the persons who exercise (207.0). Average HDL-C was influenced by drinking habits. The GT and TT alleles in ADIPOQ 45 T>G responded to drinking significantly (Fig. 1E).
Table 6. Relation between LIPE –60 C>G genetic polymorphism and clinical data.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>CC (n=132–138)</th>
<th>CG (n=10)</th>
<th>p (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>48.46±5.41</td>
<td>51.30±4.72</td>
<td>0.109</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.3±5.30</td>
<td>165.9±3.67</td>
<td>0.051</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.91±9.38</td>
<td>66.50±6.20</td>
<td>0.425</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.00±2.84</td>
<td>24.20±2.59</td>
<td>0.836</td>
</tr>
<tr>
<td>Waist circumfrence (cm)</td>
<td>84.09±6.03</td>
<td>84.40±4.84</td>
<td>0.875</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>171.4±112.4</td>
<td>99.20±52.89</td>
<td>0.046*</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>214.8±37.9</td>
<td>212.8±34.7</td>
<td>0.809</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>53.48±11.93</td>
<td>59.60±12.83</td>
<td>0.121</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>127.1±34.6</td>
<td>132.4±37.3</td>
<td>0.640</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>104.1±23.9</td>
<td>113.4±32.0</td>
<td>0.248</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.40±0.822</td>
<td>5.50±0.683</td>
<td>0.712</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.236±0.423</td>
<td>7.240±0.398</td>
<td>0.974</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.578±0.235</td>
<td>4.600±0.279</td>
<td>0.774</td>
</tr>
</tbody>
</table>

All values are means±SD. ab Different letters indicate significant difference at p<0.05 among groups by Fisher’s protected least significant difference method. Asterisk (*) indicates significant difference by one-way ANOVA.

Fig. 1. The association with genotype, clinical data and lifestyle factor in questionnaire. Questionnaires on the lifestyle behavior were answered on Likert scales. Bars represent the means±SD (n=7–84). A, B, C. As described in “Materials and Methods,” food consumption habits were indicated by 1: like it very much; 2: like it slightly; 3: do not like it; 4: do not eat it. Answer 1 and 2 were categorized “Yes” and 3 and 4 were “No.” D. Exercise habits were scaled as 1: never; 2: light; 3: hard. Answer 1 was categorized “No” and 2 and 3 were “Yes.” E. Alcohol consumption habits were scaled as 1: never drank; 2: do not drink now; 3: drink a glass of alcohol per day; 4: drink two glasses of alcohol per day; 5: drink three glasses of alcohol per day. Answer 1 and 2 were categorized “No” and 3, 4 and 5 were “Yes.”
DISCUSSION

It has been reported that obesity is a major risk factor for metabolic disorders such as diabetes mellitus, hypertension, and atherogenic diseases (1, 2). In Japanese males, obesity frequently appear in middle-aged or after that time. However, the studies among Japanese are few. Therefore, this study has focused on middle-aged Japanese men. Although our study was performed in northern Kyushu Island in Japan, the clinical markers of these subjects were similar to the average level of the Japanese male population aged 40 to 59 (11). The National Health and Nutrition Survey of 2004 in Japan has reported the following for ages 40–49 and 50–59: height was 170.4 ± 5.8 and 167.5 ± 5.7 cm respectively, weight: 69.9 ± 10.7 and 66.5 ± 9.3 kg, BMI: 24.07 ± 3.37 and 23.69 ± 2.89 kg/m², serum triglyceride: 179.3 ± 150.7 and 169.8 ± 112.9 mg/dL, TC: 211.8 ± 36.0 and 207.0 ± 38.1 mg/dL, HDL-C: 56.4 ± 16.0 and 57.2 ± 17.0 mg/dL, blood glucose level: 97.2 ± 16.6 and 104.0 ± 33.6 mg/dL, HbA1c: 5.1 ± 0.6 and 5.2 ± 1.0%, total protein: 7.3 ± 0.4 and 7.2 ± 0.4 g/dL, and albumin: 4.7 ± 0.2 and 4.6 ± 0.3 g/dL (11), which are almost the same as in our study. Consequently, it is considered that our results might reflect typical characteristic of middle-aged Japanese men.

Obesity has become a major global health problem, but its association with hereditary factors, lifestyle factors and biochemical profiles in Japanese is not understood. We have shown before that total cholesterol was significantly associated with habitual rice consumption in Tottori Prefecture in Japan (27). However not only eating habits but also genetic polymorphisms are strongly associated with obesity. SNP variations appear to be responsible for obesity, and the ADIPOQ and PLIN gene polymorphisms are emerging as potential major players in obesity (24). However, the association between ADIPOQ 45 T>G and ADIPOQ 276 G>T polymorphism with metabolic syndrome was conflicting (24). The substitution of T to G in ADIPOQ 45 T>G and G to T substitution in ADIPOQ 276 G>T have been associated with obesity in Japanese subjects, Caucasian residents of the Gargano area (east coast of Italy) and Taiwan residents (5, 6, 20); on the other hand, González-Sánchez et al. have shown no association between these polymorphisms and BMI in Spanish subjects (28). Our results indicate the SNP of ADIPOQ 45 T>G is associated with triglyceride, total cholesterol and HbA1c. Furthermore, the data for ADIPOQ 276 G>T also showed significant association between polymorphism and BMI in our study. Carriers of the GG allele had lower BMI than those of the GT allele. However, in Spanish subjects there was no association between ADIPOQ 276 G>T and BMI. In fact, in the Spanish study, there was no significance for BMI between the GG+GT allele and TT allele of ADIPOQ 276 G>T SNP, while we found a difference between the GG allele and GT allele; hence the differences between the two studies are incommensurable. However, the contributory cause of the inconsistency between our results and the Spanish subjects’ data (28) might be lifestyle in each area, under strong correlation with genotype and clinical characteristics. In most areas, the inhabitants have their own culture or climate, and lifestyle factors such as dietary and behavioral patterns are deeply entwined in their culture or climate. These factors for causal association between SNPs and clinical characteristics are overly complicated and never negligible. At the same time, Hara et al. (20) have found that 40% of Japanese have the GG allele of ADIPOQ 276 G>T, which is almost same as our data. Consequently, the validity of our data might be supported. Additionally, Kang et al. (29) have shown that triglyceride of the TT genotype of ADIPOQ 276 G>T was significantly lower than that of other genotypes in Japanese men. This result is similar to our data. The study we have shown here was performed in Japan, near the Korean peninsula. Therefore, bilateral culture or climate can have a comparatively stronger impact than nationality, which could result in this correspondence.

Indeed, lifestyle factors influenced clinical data in our study. BMI of subjects who like sweets and have the GG allele in ADIPOQ 276 G>T was higher than that of subjects who don’t like them. The subjects who have no T allele might have a susceptibility to sweets. The habit of eating fruits and fish affected LDL-C of the GT allele and HbA1c of the TT allele in ADIPOQ 276 G>T. Reiser et al. indicated that a diet high in fructose increases the levels of LDL-C (30). From our data on Japanese males, the effect was noticeable in the genotype. Bantle et al. reported that dietary fructose was associated with increased fasting and postprandial plasma triacylglycerol concentrations in men (31). However, triglyceride in our data was not related to fruit preference. Concerning the preference for fish, existence of the A allele in the subjects might be the key point. It is suggested that the value of clinical marker is affected by allele types and lifestyle behaviors. However, the study of lifestyle factors, polymorphisms and clinical markers in Japanese is well not researched. In this study, new associations among lifestyle behaviors, polymorphisms and clinical markers were found.

Exercise habits influenced total cholesterol of the GT allele at ADIPOQ 45 T>G. As concerns exercise, Huang et al. (9) have reported that plasma adiponectin concentration in genotype GT+TT at ADIPOQ 276 G>T is increased by exercise training in Japanese. The improvement of clinical marker through some exercise may be a highly effective approach for GT allele carriers of ADIPOQ 45 T>G.

Additionally, the HDL-C of subjects who drink alcohol was higher than those who don’t drink in GT and TT alleles of ADIPOQ 45 T>G. This result indicated that having the T allele in ADIPOQ 45 T>G might indicate a response to alcohol.

PLIN4 polymorphism indicated a significant association with weight, BMI and waist circumference in middle-aged Japanese men. This is the first report to demonstrate an association between the PLIN4 polymorphism and obesity in middle-aged Japanese men. The AA ge-
otype was significantly heavier than other genotypes. The same data were also reported by Qi et al. (32) and Kang et al. (29). On the other hand, Qi et al. (33) have demonstrated that the GA+AA genotype in PLIN4 results in lower body weight than the GG genotype in women of the general Spanish population. Yet, there is no correlation between the genotype and clinical data in men. The reason for this difference remains unclear; however, some lifestyle factors of Japanese might affect clinical markers synergistically with genetic factors.

The LIPE −60 C>G polymorphism was not associated with BMI but was significantly associated with triglyceride (Table 6). This polymorphism is located 60 bp upstream from the mRNA transcription starting point and is related to the LIPE transcription rate. The G construct has 38.5% lower luciferase activity compared with the C construct (34); therefore, the G allele carrier seems to have a lower LIPE protein. The LIPE knockout mouse was produced by Haemmerle et al. (35), and serum triglyceride was significantly lower in this knock-out model than in wild-type mice. The results obtained in this research match the reports by the animal experiments and in vitro transcription experiments. Therefore, the LIPE −60G allele decreased the transcription rate and, as the result, also decreased the level of serum triglycerides. We previously found that the transcription rate is subject to change due to nutritional conditions (36), and so nutrient composition in ingested foods can affect some clinical markers.

Caucasian women carriers of the −60G allele exhibited a lower percentage of body fat than noncarriers, whereas in African-American women the −60G allele was associated with higher BMI and % FAT (23). The LIPE −60 C>G polymorphism is also associated with increased waist circumference in non-obese subjects, whereas it is not associated with fasting plasma triglycerides (37). These data are not consistent with those described here. The discrepancy among these studies might be due to not only racial or sexual differences but also by lifestyle, culture, or climate. Unfortunately, no data exist regarding LIPE −60C>G SNP in the Japanese population; however, the Arg309Cys substitution in LIPE was described by Shimada et al. (38). Carriers of Cys had higher total cholesterol, but serum triglycerides did not differ.

As mentioned above, our results indicated that not only genetic polymorphism but also lifestyle factors are strongly linked to obesity, in other words, it is essential that improvement of lifestyle and genotype hit it off with each other, to prevent or improve obesity or obesity-related disorders. That can lead to a decrease of drug dose, and more importantly, unambiguous prevention from obesity or obesity-related disorders. This study is the first report that clarifies the association between ADIPOQ, PLIN and LIPE, and clinical data with lifestyle behavior in middle-aged Japanese males. It is considered that further large-scale SNPs should be analyzed with clinical marker and lifestyle factors by genome wide association study.

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Disclosure
The authors declared no conflict of interest.

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