Common variants of GIP are associated with visceral fat accumulation in Japanese adults

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Nakayama K, Watanabe K, Boonvisut S, Makishima S, Miyashita H, Iwamoto S. Common variants of GIP are associated with visceral fat accumulation in Japanese adults. Am J Physiol Gastrointest Liver Physiol 307: G1108–G1114, 2014.—Animal studies have demonstrated that glucose-dependent insulino tropic polypeptide (GIP) and GIP receptor (GIPR) contribute to the etiology of obesity. In humans, genomewide association studies have identified single nucleotide polymorphisms (SNPs) in the GIPR gene that are strongly associated with body mass index (BMI); however, it is not clear whether genetic variations in the GIP gene are involved in the development of obesity. In the current study, we assessed the impact of GIP SNPs on obesity-related traits in Japanese adults. Six tag SNPs were tested for associations with obesity-related traits in 3,013 individuals. Multiple linear regression analyses showed that rs9904288, located at the 3′-end of GIP, was significantly associated with visceral fat area (VFA). Moreover, rs1390154 and rs4794008 showed significant associations with plasma triglyceride levels and hemoglobin A1c levels, respectively. Among the significant SNPs, rs9904288 and rs1390154 were independently linked with SNPs in active enhancers of the duodenum mucosa, the main GIP-secreting tissue. The haplotypes of these two SNPs exhibited stronger associations with VFA. Numbers of VFA-increasing alleles of rs9904288 and BMI-increasing alleles of previously identified GIPR SNPs showed a strong additive effect on VFA, waist circumference, and BMI in the subject population. These novel results support the notion that the GIP-GIPR axis plays a role in the etiology of central obesity in humans, which is characterized by the accumulation of visceral fat.

GLUCOSE-DEPENDENT INSULINOTROPIC polypeptide; glucose-dependent insulino tropic polypeptide receptor; single nucleotide polymorphism

GLUCOSE-DEPENDENT INSULINOTROPIC polypeptide (GIP) is a hormone produced by K cells in the upper gastrointestinal tract, mainly in the duodenum and jejunum. Upon ingestion, GIP is secreted from K cells and induces pancreatic β-cells to release insulin (16). The action of GIP is mediated by interactions with the GIP receptor (GIPR), a G protein-coupled receptor that regulates the intracellular cyclic adenosine monophosphate signaling pathway (11). In addition to pancreatic β-cells, adipocytes and osteoblasts express functional GIPR. Studies in rodent models indicate that regulation of adiposity is an important physiological function of GIP. GIP induces glucose uptake, activity of lipoprotein lipase, and accumulation of triglycerides by 3T3-L1 adipocytes. GIPR deletion counteracts diet-induced obesity in leptin-deficient mice, and administration of a GIPR antagonist was found to suppress weight gain in mice fed a high-fat diet (10, 15, 34). Furthermore, the administration of chemically modified starch or 1-monoacylglycerols attenuates postprandial secretion of GIP and reduces visceral fat accumulation in mice fed a high-fat diet (25, 26).

Animal studies support targeting of GIP signaling as a potential new treatment for human obesity. However, the role of GIP signaling in the development of obesity in humans is still unclear. Miglitol, an α-glucosidase inhibitor, inhibits glucose uptake in the upper gastrointestinal tract in patients with type 2 diabetes. Miglitol also lowers the postprandial secretion of GIP and reduces body weight, indicating that GIP and GIPR regulate adiposity in humans (19). Genomewide association studies (GWASs) have revealed a strong association of single nucleotide polymorphisms (SNPs) located in or near the GIPR locus with the insulinogenic index and body mass index (BMI) in European (24, 27) and East Asian (20, 33) populations. In a longitudinal study, a GIPR SNP was shown to be associated with greater weight loss and improvement of glucose metabolism in overweight and obese subjects administered low-fat diets (21).

To treat obesity by inhibiting GIP signaling, it is necessary to collect further evidence of the involvement of GIP and GIPR in the etiology of obesity. SNPs in or near GIP are associated with blood clinical parameters and plasma levels of GIP, but not with obesity traits (4, 5, 29). Although recent advances in GWASs have identified multiple genes, including GIPR, that are associated with human obesity (7), the genetic architecture underlying depot-specific adiposity remains unclear. Visceral adipose tissue (VAT) is of medical interest because of its capacity to exacerbate metabolic abnormalities. Limited numbers of genetic variants have been reported for the specific control of VAT area (8, 18). Expression levels of GIPR are higher in VAT than in subcutaneous adipose tissue, suggesting that genetic variants of GIP may affect visceral fat accumulation rather than whole body adiposity (22).

In the present study, SNPs in/near GIP were tested for association with obesity-related traits, especially with visceral fat area (VFA), as measured with the bioelectrical impedance (BI) method.

MATERIALS AND METHODS

Subjects. The study analyzed 3,013 Japanese individuals. Details of the population were described previously (18). Briefly, participants were enrolled during attendance at general health checkups at the Jichi Medical University Hospital from January 2009 to March 2011. All participants provided written informed consent. VFA at the umbilical level was measured by the BI method (23), which is guaranteed to measure VFA as accurately as the computed tomography (CT) scan...
method (23). In a small portion of our population (n = 272), VFA measured by the BI method was highly correlated with values measured by the CT scan method (correlation coefficient = 0.88, \( P < 0.001 \)). BMI, waist circumference (WC), fasting plasma glucose levels (mg/dl), hemoglobin A_{1c} (HbA_{1c}) levels, and plasma triglyceride levels (mg/dl) were also measured in all participants. Characteristics of the individuals in this study are summarized in Table 1. The design of the present study was approved by the ethical committees of Jichi Medical University.

### RESULTS

A common variant in the proximity of GIP was associated with VFA. The identified GIP SNPs had no significant deviation from the HWE (\( P > 0.05 \)). In the results of multiple linear regression analyses, rs9904288, located downstream of GIP, showed a significant association with VFA (\( P = 0.0055 \), Table 2). Adjusted mean values of VFA and 95% confidence intervals (CI) of rs9904288 genotypes were as follows: TT = 89.7 (88.2–91.2) cm²; TC = 92.1 (89.9–94.4) cm²; and CC = 98.4 (91.9–105.0) cm². Carriers of the rs9904288 C allele also exhibited a tendency toward increased WC and BMI, but these associations were not observed following multiple testing cor-

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**Table 1. Characteristics of the subjects**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals</td>
<td>1,572</td>
<td>1,339</td>
</tr>
<tr>
<td>Age, yr</td>
<td>52.56 (9.32)</td>
<td>50.49 (8.67)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.31 (3.19)</td>
<td>22.65 (3.57)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>86.39 (8.49)</td>
<td>80.87 (9.26)</td>
</tr>
<tr>
<td>Visceral fat area, cm²</td>
<td>114.33 (41.90)</td>
<td>62.48 (26.91)</td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>105.08 (20.26)</td>
<td>95.05 (13.04)</td>
</tr>
<tr>
<td>Hemoglobin A_{1c}, %</td>
<td>5.3 (0.65)</td>
<td>5.2 (0.47)</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>132.54 (88.33)</td>
<td>87.46 (47.13)</td>
</tr>
<tr>
<td>Medications for diabetes, %</td>
<td>4.02</td>
<td>3.58</td>
</tr>
<tr>
<td>Medications for dyslipidemia, %</td>
<td>10.11</td>
<td>11.42</td>
</tr>
</tbody>
</table>

Means and SD (in parenthesis) are shown for each quantitative trait. Data are based on 2,911 individuals without abdominal surgery.
GIP polymorphisms and visceral fat accumulation

**Table 3. Pairwise linkage disequilibrium status of GIP SNPs**

<table>
<thead>
<tr>
<th>rs12941604</th>
<th>rs9904288</th>
<th>rs2291725</th>
<th>rs4794008</th>
<th>rs1390154</th>
<th>rs11650936</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12941604</td>
<td>0.932</td>
<td>0.991</td>
<td>0.097</td>
<td>0.185</td>
<td>0.108</td>
</tr>
<tr>
<td>rs9904288</td>
<td>0.013</td>
<td>0.982</td>
<td>0.048</td>
<td>0.015</td>
<td>0.350</td>
</tr>
<tr>
<td>rs2291725</td>
<td>0.196</td>
<td>0.602</td>
<td>0.096</td>
<td>0.434</td>
<td>0.038</td>
</tr>
<tr>
<td>rs4794008</td>
<td>0.003</td>
<td>0.002</td>
<td>0.007</td>
<td>0.066</td>
<td>0.745</td>
</tr>
<tr>
<td>rs1390154</td>
<td>0.001</td>
<td>0.194</td>
<td>0.138</td>
<td>0.002</td>
<td>0.998</td>
</tr>
<tr>
<td>rs11650936</td>
<td>0.005</td>
<td>0.005</td>
<td>0.387</td>
<td>0.084</td>
<td></td>
</tr>
</tbody>
</table>

Upper and lower triangular matrices indicate pairwise $D'$ values and $r^2$ values, respectively.

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rs1390154 and rs11650936 tended to influence VFA ($P = 0.040$ and 0.030, respectively).

**Fig. 1.** Functional annotation of the glucose-dependent insulinotropic polypeptide (GIP) single nucleotide polymorphisms (SNPs) showing significant associations. *SNPs showing associations with metabolic traits. SNPs in strong linkage disequilibrium status with one of the associated SNPs ($r^2 > 0.8$) are also shown (grouped by column). 1Abbreviations of annotation are as follows: IN, intronic; SN, synonymous polymorphism; U3, 3'-untranslated region; dbSNP, database of single nucleotide polymorphisms; GERP, genomic evolutionary rate profiling; SiPhy, site-specific phylogenetic analysis. 2SNPs located in an evolutionary conserved region are highlighted. 3The no. of epigenomic experiments reporting enhancer activity is indicated. 4SNPs located in putative binding motifs of transcription factors are highlighted.
affecting the transcription and translation of GIP. Similarly, the triglyceride-associated SNP rs1390154 was in LD with an SNP in a putative enhancer of the duodenum mucosa.

**Haplotype association test.** rs9904288 and rs1390154 were in incomplete LD ($r^2 = 0.194$ and $D' = 0.651$, Table 3) and showed a similar pattern of association with metabolic parameters (increasing VFA, WC, and plasma triglyceride levels), suggesting that haplotype analysis might refine the association with the metabolic parameters. Among the statistically inferred haplotypes, the VFA- and triglyceride-increasing alleles of SNPs rs9904288 and rs1390154 showed refinement of associations with VFA ($P = 0.0014$), WC ($P = 0.0065$), and BMI ($P = 0.014$). The adjusted mean VFA and 95% CIs of diplootypes were as follows: CA/CA = 101.1 (92.5–109.7) cm², CA/others = 92.9 (90.5–95.4) cm², and others/others = 98.4 (88.2–91.1) cm². The association with triglyceride levels was marginal ($P = 0.01$). The HbA1c-associated SNP rs4794008 was not linked with enhancer elements in organs or tissues expressing GIP. SNPs not exhibiting any association, such as rs12941604, rs2291725, rs11650936, and 45 tightly linked variants, did not localize in the enhancer of the duodenum mucosa (Supplemental Table 1).

**Additive effects of GIP and GIPR SNPs on VFA.** The genotypes of GIPR SNPs discovered by GWASs for BMI (20, 27, 33) were tested for association with obesity traits in our population (Table 4). No significant deviation from the HWE was observed. In accordance with the strong correlation among the obesity traits, obesity risk alleles identified by GWAS showed a tendency to increase WC and VFA, although the association was not observed when data were analyzed with our multiple testing correction method ($P > 0.00625$). The tested GIPR SNPs were not in strong LD in East Asian individuals in the 1000 Genome project and in our population ($r^2 = 0.01$, $D' = 0.232$), suggesting that rs2287019 and rs55669001 might have independent functional consequences and may act additively to cause body fat accumulation. Linear regression models accounting for the number of VFA-increasing alleles of SNPs rs2287019 and rs55669001 (0, 1, 2, 3, and 4) were strongly associated with VFA ($P = 0.0034$), WC ($P = 0.001$), and BMI ($P = 0.005$). In contrast, haplotype trend regression analysis did not refine the associations (Table 4). GIP and GIPR may affect the same signaling pathway to regulate body fat; therefore, variants of both genes may show an additive effect on visceral fat accumulation. A multiple linear regression model that included the sum of VFA-increasing alleles of GIP rs9904288, GIPR rs2287019, and rs55669001 (0–6) was tested. A number of risk alleles correlated significantly with VFA ($P = 0.000084$, Fig. 2), WC ($P = 0.000091$), and BMI ($P = 0.00053$).

**DISCUSSION**

The present study tested the hypothesis that a genetic association exists between GIP and visceral fat accumulation in Japanese adults. A common SNP located near GIP, rs9904288, was associated significantly with VFA and in linkage disequilibrium with an SNP proximal to an enhancer element active in the duodenum mucosa. Moreover, rs9904288 showed additive effects with the GWAS-discovered GIPR variants (rs2287019 and rs55669001) on obesity traits, such as VFA, WC, and BMI. These results provided new evidence supporting the roles of GIP and GIPR in the development of obesity in humans. rs9904288 and the relevant haplotype showed a stronger association with VFA than with BMI, suggesting that the obesity-inducing action of GIP had a more profound impact on depot-specific adiposity than on whole body adiposity. Consistently, the additive effects of GIP and GIPR variants on

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**Table 4. Association analyses of GIPR SNPs**

<table>
<thead>
<tr>
<th>dbSNP ID</th>
<th>Position in Chr 19</th>
<th>Feature</th>
<th>Alleles</th>
<th>Frequency</th>
<th>HWE</th>
<th>VFA</th>
<th>WC</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs55669001</td>
<td>46177235</td>
<td>GIPR Intron</td>
<td>T/C</td>
<td>0.439</td>
<td>0.421</td>
<td>0.029</td>
<td>0.035</td>
<td>0.046</td>
</tr>
<tr>
<td>rs2287019</td>
<td>46202172</td>
<td>QPTCL Intron</td>
<td>C/T</td>
<td>0.789</td>
<td>0.963</td>
<td>0.031</td>
<td>0.023</td>
<td>0.038</td>
</tr>
</tbody>
</table>

**Haplotypes**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>VFA</th>
<th>WC</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.398</td>
<td>0.034</td>
<td>0.015</td>
</tr>
<tr>
<td>CC</td>
<td>0.391</td>
<td>-0.006</td>
<td>0.685</td>
</tr>
<tr>
<td>CT</td>
<td>0.171</td>
<td>-0.032</td>
<td>0.021</td>
</tr>
<tr>
<td>TT</td>
<td>0.040</td>
<td>-0.006</td>
<td>0.664</td>
</tr>
</tbody>
</table>

**Discussion**

The present study tested the hypothesis that a genetic association exists between GIP and visceral fat accumulation in Japanese adults. A common SNP located near GIP, rs9904288, was associated significantly with VFA and in linkage disequilibrium with an SNP proximal to an enhancer element active in the duodenum mucosa. Moreover, rs9904288 showed additive effects with the GWAS-discovered GIPR variants (rs2287019 and rs55669001) on obesity traits, such as VFA, WC, and BMI. These results provided new evidence supporting the roles of GIP and GIPR in the development of obesity in humans. rs9904288 and the relevant haplotype showed a stronger association with VFA than with BMI, suggesting that the obesity-inducing action of GIP had a more profound impact on depot-specific adiposity than on whole body adiposity. Consistently, the additive effects of GIP and GIPR variants on visceral fat accumulation were observed.

**Figure 2.** Additive effects of GIP and GIP receptor (GIPR) SNPs on visceral fat area (VFA). Bars indicate the no. of individuals in each risk-score category (sum of the no. of VFA-increasing alleles of GIP rs9904288, GIPR rs2287019, and rs55669001). Plots and error bars indicate means and SEs, respectively, of VFA in each risk-score category.
VFA or WC were stronger than the effects on BMI. However, the molecular and cellular mechanisms determining the effects of these SNPs on depot-specific adiposity are still unknown. In obese human subjects, insulin resistance reduces GIPR expression and GIP activity in subcutaneous adipose tissue but not in VAT (3, 22). It is possible that GIP contributes to obesity by acting on VAT, despite the fact that, under conditions of insulin resistance, GIP signaling in subcutaneous adipose tissue is blunted. As a consequence, the genetic variants of the GIP-GIPR axis exhibit stronger associations with VFA and WC, and obesity traits are influenced by expansion of VATs.

We also observed associations between GIP SNPs and obesity-related blood parameters. rs1390154 was associated with fasting plasma triglyceride levels. Direct involvement of the GIP-GIPR axis in liver lipid metabolism, development of fatty liver disease, and regulation of plasma lipid levels was observed in animal models and human clinical studies (1, 14, 17). In contrast, the multiple linear regression model employed in the present study suggested that the association of GIP SNPs with triglyceride levels might reflect the effects of GIP variants on visceral fat accumulation. It is conceivable that visceral fat accumulation increases free fatty acid supply to the portal vein, leading to enhanced hepatic lipogenesis and secretion of very-low-density lipoprotein. The association between rs4794800 and HbA1c levels remained significant after adjustment for VFA. In obese mice, long-term administration of a GIPR antagonist significantly decreased HbA1c levels (14). Although GIP variants might affect glucose homeostasis, it is not clear why the flanking region of rs4794800 did not show chromatin status changes in enhancer element of tissues or organs that express GIP. Moreover, it was not reported whether this SNP affects insulin response to oral glucose challenge.

Chang et al. reported that SNPs rs3895874, rs3848460, and rs937301 in the putative GIP promoter region might affect in vitro transcriptional activity in HEK293 cells and serum GIP and glucose levels in pregnant women (4). These SNPs were in almost absolute LD with rs2291725 in the HapMap JPT+CHB (r² = 0.97). In the current study, rs2291725 did not show association with any metabolic parameters, including fasting plasma glucose levels. This discrepancy may be explained by the fact that Chang et al. tested pregnant women, whereas pregnant women were excluded in our study.

The main limitation of the current study is the lack of replication analysis for GIP. We unfortunately do not have an independent cohort with a sufficiently large sample size. We hope that the present findings will be validated in cooperation with other research groups in the future. Association of rs9904288 and abdominal subcutaneous fat area should be tested to clarify the depot-specific effects of the GIP SNP. Moreover, the effects of GIP SNPs on postprandial glucose levels should be investigated since GIP secretion increases with feeding. A recent study reported that a functional non-synonymous variant of GIPR decreased bone mineral density and increased the risk of fracture in perimenopausal women (31). Therefore, testing the impact of GIP SNPs on bone mineral density and fracture may provide additional support for the role of the GIP-GIPR axis in bone metabolism.

In summary, we discovered a common GIP genetic variant that influenced VFA in Japanese adults and provided new evidence supporting the crucial role of the GIP-GIPR axis in the development of central obesity and related diseases in humans.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS
K.N. and S.I. conception and design of research; K.N., K.W., S.B., and S.M. performed experiments; K.N. and H.M. analyzed data; K.N. and H.M. interpreted results of experiments; K.N. prepared figures; K.N. drafted manuscript; K.N., K.W., S.B., S.M., H.M., and S.I. approved final version of manuscript.

REFERENCES


