ORIGINAL ARTICLE

# Association of protein tyrosine phosphatase 1B gene polymorphism with the effects of weight reduction therapy on bodyweight and glycolipid profiles in obese patients

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## ABSTRACT

**Aims/Introduction:** Weight reduction therapy is the primary treatment to prevent complications of obesity, such as lifestyle diseases and cardiovascular disease; however, to date, useful methods and genetic factors for predicting the outcomes of weight reduction therapy in obese patients have not been established. Protein tyrosine phosphatase 1B (PTP1B), a negative regulator for insulin and leptin signaling, potentially modulates glucose and energy homeostasis. This study aimed to investigate the contribution of *PTPN1* polymorphisms on weight reduction and diabetes in obese Japanese patients.

**Materials and Methods:** *PTPN1*-tagged single-nucleotide polymorphisms (SNPs) rs3787348 and rs6067484 were genotyped in 447 obese Japanese patients from the general population. In this prospective cohort study, all obese patients underwent a 3-month weight reduction therapy with lifestyle modifications, as recommended by guidelines.

**Results:** In obese patients (male/female 196/251, age 50 ± 15 years, body mass index [BMI] 32 ± 6 kg/m$^2$), the minor allele appeared at a frequency of 45.5% in rs3787348 SNP of the *PTPN1* gene. The T allele of rs3787348 was significantly associated with a higher BMI ($P = 0.041$ in the additive model). The patients with the T allele in SNP rs3787348 of *PTPN1* had significantly smaller reductions in BMI, bodyweight and waist circumference levels during weight reduction therapy (BMI G/G, −1.9 ± 0.2; G/T, −1.5 ± 0.1; T/T, −1.2 ± 0.1; $P = 0.001$ in the additive model).

**Conclusions:** Our findings show that the SNP rs3787348 in *PTPN1* was associated with the effects of weight reduction therapy on BMI and waist circumference among obese Japanese patients.

## INTRODUCTION

Obesity is a worldwide issue and a major risk factor for cardiovascular disease (CVD), dyslipidemia, hypertension, insulin resistance, type 2 diabetes and cancer[1]. Weight reduction therapy is essential for the management of obesity, and can decrease obesity-related metabolic sequelae and CVD complications[2,3]. We previously reported the beneficial effects of short-term weight reduction with diet and exercise guidance on arterial stiffness, serum oxidative LDL, and cystatin C, all of which are CVD and chronic kidney disease risk factors[4–6].

Genetic factors contribute to the development of obesity, and to date, several candidate gene studies and genome-wide association studies using observational cohorts have shown that hundreds of genetic variants and loci are associated with body mass index (BMI)[7,8]. In a recent genome-wide association study

of BMI in a Japanese population, 85 loci were found to be significantly associated with obesity[9]. However, the contribution of genetic variants to efficient weight loss in response to weight reduction therapy remains unknown.

The ubiquitously expressed protein tyrosine phosphatase 1B (PTP1B), encoded by the *PTPN1* gene, dephosphorylates phosphotyrosine residues of the active insulin receptor, which consequently downregulates insulin signaling[10]. PTP1B also inhibits leptin signaling through the dephosphorylation of Janus kinase 2 and signal transducer and activator of transcription 3[11]. The disruption of the *PTPN1* gene in mice results in increased insulin sensitivity and resistance to diet-induced obesity, and enables the normalization of blood glucose levels[12,13]. The *in vitro* inhibition of PTP1B improves adipocyte energy storage and insulin sensitivity, and the PTP1B inhibitor provides a prolonged reduction in bodyweight (BW) and glycemic parameters in overweight patients with type 2 diabetes[14–16]. Taken together, these data illustrate the crucial role of PTP1B in the insulin and leptin pathways, and show that abnormal PTP1B activity might lead to insulin resistance, and thereby to type 2 diabetes and obesity.

In humans, *PTPN1* maps to chromosome 20q13.13, which also contains a major human quantitative trait locus for obesity and type 2 diabetes, thus further supporting *PTPN1* candidacy in type 2 diabetes and obesity[17,18]. Several previous studies have found convincing associations of genetic variants of *PTPN1* with type 2 diabetes in two independent studies of white Americans, and with insulin sensitivity and fasting glucose in Hispanic Americans[19,20]. However, a meta-analysis involving the largest number of European case–control samples was unable to replicate this association for any single SNP or haplotype[21]. In addition, several previous studies have shown that *PTPN1* variants were associated with obesity in French people[22,23]. However, among Pima Indians, SNPs within *PTPN1* were unlikely to have a major role in the etiology of type 2 diabetes or obesity[24]. Accordingly, the results of the association of *PTPN1* polymorphisms with diabetes and obesity differ slightly in different populations, and no studies have yet reported on the contributions of *PTPN1* gene polymorphisms on obesity and diabetes in Japanese populations. Furthermore, although weight reduction therapy is essential for the management of obesity, little is known about the genetic contribution to weight reduction therapy in obese individuals, and no studies have shown whether *PTPN1* genotypes affect longitudinal changes in BW and glycolipid metabolism during weight reduction therapy in obese patients. The present study aimed to determine the association of *PTPN1* gene polymorphisms with weight reduction and the risk of diabetes in obese Japanese patients.

## MATERIALS AND METHODS
### Study design
In the present prospective cohort study, 447 overweight or obese Japanese outpatients were consecutively enrolled in the outpatient clinic at the National Hospital Organization Kyoto Medical Center during the period from April 2005 to March 2011. Obese patients were defined as those with a BMI >25 kg/m², based on the guidelines of the Japan Society for the Study of Obesity (JASSO)[6,25]. The exclusion criteria were as follows: a previous history of CVD; other vascular diseases; apparent renal disease; severe liver dysfunction; or secondary obesity due to endocrine disorders, such as Cushing syndrome, polycystic ovary syndrome, acromegaly and hypothyroidism. None of the patients had received anti-obesity drugs. The study protocol was approved by the ethics committee for human research at the Kyoto Medical Center (approval number: 05-27, 09-10), and all participants provided written informed consent. This study adhered to the STROBE checklist (Appendix S1).

### Data collection and laboratory measurements
Height and BW were measured, and BMI was calculated as the weight in kilograms divided by height in meters squared; BMI was used as an index of obesity. The primary outcome in this study was BMI. Waist circumference (WC) was measured to the nearest 0.1 cm at the level of the umbilicus with participants in the standing position at the end of expiration while breathing gently[3–5]. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were each measured twice with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Komaki, Japan). Blood was obtained from the antecubital vein in the morning after an overnight fast, and was used to determine fasting plasma glucose (FPG), glycosylated hemoglobin A1c (HbA1c; National Glycohemoglobin Standardization Program), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels according to standard procedures[3–6]. Immunoreactive insulin (IRI) was measured with an enzyme immunoassay (Tosoh, Tokyo, Japan)[3–5]. The insulin resistance index was assessed by the homeostasis model assessment (HOMA-R)[4]. Serum levels of leptin and adiponectin were determined as previously described. The adiponectin levels were measured with enzyme-linked immunosorbent assays (Otsuka Pharmaceutical, Tokyo, Japan). The leptin levels were determined using a radioimmunoassay (Linco Research, St. Charles, MO, USA)[3–5].

### DNA preparation and SNP genotyping
Genomic DNA was prepared using BioRobot EZ1 (QIAGEN, Valencia, CA, USA) from blood samples collected from each participant. High-throughput genotyping of the *PTPN1*-tagged SNPs (rs3787348 and rs6067484) was carried out using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). We selected two *PTPN1*-tagged SNPs (rs3787348 and rs6067484) with reference to the study by NJ Spencer-Jones *et al.*[26] The polymerase chain reaction primers and TaqMan probes were purchased from Applied Biosystems and optimized according to the manufacturer's protocol[27]. Water blanks were installed on each plate and evaluated as a negative control to guarantee accuracy.

### Weight reduction therapy

All obese patients were subjected to weight reduction therapy through lifestyle modifications, which included reducing energy intake and increasing physical activity for 3 months[3–5]. All patients who underwent weight reduction therapy were instructed to maintain the same levels of energy intake and physical activity for the entire period, as recommended by the Japan Atherosclerosis Society's Guidelines for the Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases[3–6,28]. The diet therapy prescribed consisted of 25 kcal/kg of ideal BW per day. The participants consumed a diet in which carbohydrates comprised 60% of the total energy intake, fats comprised 20–25% and protein comprised 15–20%. They were also instructed to exercise for >30 min at a moderate intensity at least 3 days/week. In the present study, all patients were instructed to follow our weight reduction protocol throughout the entire 3-month treatment period. To ensure adherence to the weight reduction therapy during this period, we interviewed and instructed all the patients on our weight reduction protocol, and checked the patients' daily records in our outpatient clinic every month. We measured the metabolic parameters for each patient before and after the 3-month weight reduction therapy. Smoking habits and prescribed drugs were not altered[3].

### Statistical analysis

To observe the difference in the mean change in BMI between the *PTPN1*-tagged SNPs (ts3787348), and based on information from prior reports describing the respective genotype proportions (approximately 30% of participants were G/G, 50% were G/T and 20% were T/T)[20], −2.0 and −1.0 (standard deviation 2.5 kg/m²) in G/G and T/T obese patients in change of BMI, the sample size was estimated at 447 participants assuming an alpha error of 0.05 and a beta error of 0.20 (power = 0.80).

The data are presented as the mean ± standard deviation and standard error, and $P < 0.05$ was considered statistically significant. A two-tailed, paired *t*-test was applied to evaluate the changes in conditions from baseline to those after the 3-month weight loss intervention. If the data were not normally distributed, the Wilcoxon signed-rank test was used. Changes from baseline conditions to those at 3 months were abbreviated as Δ. A general linear model analysis with an additive model was carried out to test for associations between the *PTPN1* genotypes and each parameter after adjusting for age and sex. When investigating the amount of change, a general linear model analysis was carried out to test for associations between the *PTPN1* genotypes and the change in each parameter after the weight reduction therapy with adjustment for age, sex, and the baseline value of dependent variables. When the dependent variable was a lognormally distributed variable in the general linear model analysis, the logarithmic transformation value was used. In the analysis of secondary outcomes excluding BMI (primary outcome), the Benjamini–Hochberg procedure was used to control the false positive rate in multiple comparisons and *q*-values were calculated. The number of correction targets

was calculated by multiplying the number of secondary evaluation items by the number of SNPs. All statistical analyses were carried out using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Baseline characteristics

The clinical characteristics of the patients at baseline are shown in Table 1. In the 447 obese Japanese participants (male/female 196/251, age 50.0 ± 14.0 years, BMI 32.1 ± 6.0 kg/m²), the minor allele frequencies of the *PTPN1* gene were 45.5% in the rs3787348 SNP (T allele) and 9.1% in the rs6067484 SNP (G allele). The detection success rate by genotyping was 99.3% for rs3787348 and 98.7% for rs6067484. None of the loci deviated from the Hardy–Weinberg equilibrium (rs3787348 [$P = 0.635$], rs6067484 [$P = 0.562$]).

### Association analysis of the *PTPN1*-tagged SNPs with metabolic parameters

Table 2 presents the results of the association analysis between the *PTPN1*-tagged SNPs of rs3787348 and the metabolic parameters, adjusted for age and sex. The T allele of rs3787348 was significantly associated with a higher BMI (mean ± standard error: G/G, 31.0 ± 0.6; G/T, 31.8 ± 0.3; T/T, 32.6 ± 0.4 kg/m²; $P = 0.041$ in the additive model). The T allele of rs3787348 tended to be associated with increased HbA1c levels, but this trend was not significant (G/G, 6.3 ± 0.1; G/T, 6.5 ± 0.1; T/T, 6.7 ± 0.1%; $P = 0.064$, *q*-value >0.999 in the additive model). However, the T allele of rs3787348 did not show any significant associations with BW, WC, SBP, DBP, FPG, IRI, HOMA-R, TC, TG, HDL-C, LDL-C, leptin or adiponectin (Table 2).

Table S1 presents the results of the association analysis of the tagged SNP of rs6067484 with metabolic parameters, adjusted for age and sex. No significant association was observed for rs6067484.

### Effects of bodyweight reduction therapy

Table 1 presents the characteristics of the 447 obese patients who underwent weight reduction therapy at baseline and at 3 months. Of the 447 patients, 248 (55.5%) successfully reduced their BW by >3%. BW, BMI, WC, SBP, DBP, FPG, HbA1c, IRI, HOMA-R, TC, TG, LDL-C and leptin were significantly decreased 3 months after the weight reduction therapy ($P < 0.05$). HDL-C and adiponectin levels were significantly increased 3 months after the weight reduction therapy ($P < 0.01$; Table 1). The use of prescribed drugs was not altered.

### Association analysis of *PTPN1*-tagged SNPs with changes in metabolic parameters after weight reduction therapy

Table 3 presents the results of the association analysis of the *PTPN1*-tagged SNPs (rs3787348) with the changes in metabolic parameters adjusted for age, sex and baseline value of

**Table 1** | Baseline clinical characteristics of obese patients

| | Baseline | 3-month | P-value |
|---|---|---|---|
| *n* | 447 | | |
| Sex (male/female) | 196/251 | | |
| Age (years) | 50.0 ± 14.0 | | |
| Bodyweight (kg) | 84.5 ± 19.5 | 80.8 ± 18.9 | <0.001 |
| BMI (kg/m$^2$) | 32.1 ± 6.0 | 30.7 ± 5.8 | <0.001 |
| Waist circumference (cm) | 103.1 ± 14.0 | 99.5 ± 13.2 | <0.001 |
| Systolic blood pressure (mmHg) | 142.2 ± 19.3 | 135.1 ± 16.7 | <0.001 |
| Diastolic blood pressure (mmHg) | 85.2 ± 12.5 | 81.8 ± 10.9 | <0.001 |
| Fasting plasma glucose (mmol/L) | 6.6 ± 2.2 | 6.2 ± 1.8 | <0.001 |
| HbA1c (%) | 6.5 ± 1.3 | 6.2 ± 1.0 | <0.001 |
| HbA1c (mmol/mol) | 47.5 ± 14.2 | 44.2 ± 10.9 | |
| IRI (pmol/L) | 86 (53–155) | 79 (46–145) | 0.004 |
| HOMA-R | 3.9 (2.2–8.1) | 3.5 (1.8–7.2) | 0.001 |
| Total cholesterol (mmol/L) | 5.4 ± 1.0 | 5.1 ± 0.9 | <0.001 |
| Triglyceride (mmol/L) | 1.7 (1.2–2.5) | 1.3 (0.9–1.9) | <0.001 |
| HDL cholesterol (mmol/L) | 1.4 ± 0.4 | 1.5 ± 0.4 | 0.003 |
| LDL cholesterol (mmol/L) | 3.3 ± 0.8 | 3.0 ± 0.6 | <0.001 |
| Leptin (ng/mL) | 13.2 (6.8–23.1) | 10.9 (5.7–18.3) | <0.001 |
| Adiponectin (μg/mL) | 6.1 (4.5–9.3) | 6.6 (4.8–9.5) | 0.004 |
| Proportion (*n*, %) | | | |
|   With hypertension | 286, 64.0% | | |
|   With diabetes | 173, 38.7% | | |
|   With dyslipidemia | 334, 74.7% | | |
|   Taking calcium antagonist | 80, 17.9% | | |
|   Taking ACE/ARB | 103, 23.0% | | |
|   Taking antidiabetic medication | 106, 23.7% | | |
|   Taking statins | 99, 22.1% | | |
| rs3787348 (*n*, %) | | | |
|   G/G | 90, 20.1% | | |
|   G/T | 227, 50.8% | | |
|   T/T | 130, 29.1% | | |
|   Minor allele frequency (%) | 45.5 | | |
| rs6067484, *n* (%) | | | |
|   A/A | 368, 82.3% | | |
|   A/G | 77, 17.2% | | |
|   G/G | 2, 0.5% | | |
|   Minor allele frequency (%) | 9.1 | | |

Data are expressed as the mean ± standard deviation, median (interquartile range), or the number and percentage of patients. ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment ratio; IRI, immunoreactive insulin; LDL, low-density lipoprotein.

dependent variables. After the 3-month weight reduction therapy, patients with the T allele in rs3787348 showed less significant decreased levels of BMI, BW and WC (ΔBMI: G/G, −1.9 ± 0.2; G/T, −1.5 ± 0.1; T/T, −1.2 ± 0.1 kg/m$^2$, $P = 0.001$;

ΔBW: G/G, −5.1 ± 0.5; G/T, −4.1 ± 0.2; T/T, −3.1 ± 0.3 kg; $P = 0.001$, *q*-value = 0.014; ΔWC: G/G, −5.2 ± 0.7; G/T, −4.1 ± 0.4; T/T, −2.9 ± 0.4 cm; $P < 0.001$, *q*-value = 0.013). Additionally, patients with the T allele in rs3787348 showed nominal associations with decreased levels of TC and leptin (ΔTC: G/G, −0.4 ± 0.1; G/T, −0.3 ± 0.1; T/T, −0.2 ± 0.1 mmol/L; $P = 0.034$, *q*-value = 0.238; Δleptin: G/G, −3.9 ± 0.7; G/T, −3.4 ± 0.8; T/T, −1.1 ± 0.8 ng/mL; $P = 0.011$, *q*-value = 0.103 in the additive model). The T allele of rs3787348 was associated with a trend toward reduced lowering of FPG, HbA1c and LDL-C, although this trend was not statistically significant. Conversely, the T allele of rs3787348 did not show any significant associations with ΔSBP, ΔDBP, ΔIRI, ΔHOMA-R, ΔTG, ΔHDL-C and Δadiponectin (Table 3).

Table 4 represents the results of the association analysis of the *PTPN1*-tagged SNPs (rs6067484). The A allele in rs6067484 was only nominally associated with reduced lowering of levels of WC (ΔWC: A/A, −4.0 ± 0.4; A/G, −2.2 ± 0.7; G/G, −0.5 ± 1.5 cm; $P = 0.030$, *q*-value = 0.210 in the additive model; Table 4). However, the A allele of rs6067484 did not show any significant associations with other parameter changes.

## DISCUSSION

In the present study, we found that the *PTPN1* polymorphism rs3787348 was significantly related to the beneficial effects in weight reduction and obesity-related metabolic changes (WC, TC and leptin levels) through weight reduction therapy in obese Japanese patients. This is the first report of *PTPN1* SNPs in Japanese individuals, and it is also the first report examining the association of rs3787348 in the *PTPN1* gene with obesity and weight loss. The detection of gene polymorphisms involved in weight loss and obesity-related metabolic changes might help identify specific targets for nutritional interventions to achieve significant weight loss[29].

Initially, in obese patients, the T allele of rs3787348 in *PTPN1* is significantly associated with a higher BMI; however, no significant association was observed in the SNP rs6067484 in *PTPN1*. The *PTPN1* gene encodes PTP1B, which is an enzyme that negatively regulates the signaling pathways of insulin and leptin, two hormones that are involved in the central regulation of energy balance[30]. Thus, evidence has accumulated reflecting a crucial role of PTP1B in the insulin and leptin pathways, which suggests that abnormal PTP1B activity could lead to insulin resistance, and thus to type 2 diabetes and obesity[12–16]. In humans, the *PTPN1* gene is located on 20q13, a locus that is linked to type 2 diabetes and obesity[18,19]. The present results showed that the T allele of rs3787348 in *PTPN1* was significantly associated with a higher BMI. Previous case–control studies of French individuals reported that other SNPs of the *PTPN1* gene, which differed from the SNP rs378748 that we examined, were associated with obesity[22,23]. Thus far, there have been no reports on significant associations of rs3787348 in *PTPN1* with obesity; therefore, the present study is the first report on a significant relationship between the *PTPN1* SNP

**Table 2** | Association analysis of *PTPN1* tag single-nucleotide polymorphism of rs3787348 with baseline metabolic parameters

| | rs3787348 | | | *P*-value | *q*-value |
|---|---|---|---|---|---|
| | G/G | G/T | T/T | | |
| Sex (male/female) | 36/54 | 96/131 | 64/66 | – | – |
| Age (years) | 48.1 ± 1.4 | 50.0 ± 0.9 | 51.3 ± 1.2 | – | – |
| BMI (kg/m$^2$) | 31.0 ± 0.6 | 31.8 ± 0.3 | 32.6 ± 0.4 | 0.041 | – |
| Bodyweight (kg) | 82.5 ± 1.7 | 84.0 ± 0.8 | 85.5 ± 1.0 | 0.163 | >0.999 |
| Waist circumference (cm) | 101.3 ± 1.5 | 102.6 ± 0.7 | 103.9 ± 0.9 | 0.165 | >0.999 |
| Systolic blood pressure (mmHg) | 142.2 ± 2.1 | 142.2 ± 1.0 | 142.3 ± 1.3 | 0.971 | 0.971 |
| Diastolic blood pressure (mmHg) | 85.0 ± 1.3 | 85.2 ± 0.7 | 85.4 ± 0.8 | 0.828 | 0.927 |
| Fasting plasma glucose (mmol/L) | 6.4 ± 0.3 | 6.6 ± 0.1 | 6.7 ± 0.2 | 0.357 | 0.833 |
| HbA1c (%) | 6.3 ± 0.1 | 6.5 ± 0.1 | 6.7 ± 0.1 | 0.064 | >0.999 |
| HbA1c (mmol/mol) | 45.3 ± 1.0 | 47.5 ± 1.0 | 49.7 ± 1.1 | – | |
| ln IRI (pmol/L) | 4.7 ± 0.2 | 4.6 ± 0.2 | 4.5 ± 0.2 | 0.157 | >0.999 |
| ln HOMA-R | 1.6 ± 0.1 | 1.4 ± 0.1 | 1.5 ± 0.1 | 0.809 | 0.944 |
| Total cholesterol (mmol/L) | 5.4 ± 0.1 | 5.4 ± 0.1 | 5.4 ± 0.1 | 0.550 | 0.906 |
| Triglyceride (mmol/L) | 2.0 ± 0.1 | 2.1 ± 0.1 | 2.1 ± 0.1 | 0.867 | 0.934 |
| HDL cholesterol (mmol/L) | 1.4 ± 0.03 | 1.4 ± 0.02 | 1.5 ± 0.02 | 0.220 | 0.880 |
| LDL cholesterol (mmol/L) | 3.3 ± 0.1 | 3.3 ± 0.1 | 3.3 ± 0.1 | 0.805 | 0.980 |
| ln Leptin (ng/mL) | 2.50 ± 0.06 | 2.51 ± 0.08 | 2.55 ± 0.08 | 0.270 | 0.945 |
| ln Adiponectin (μg/mL) | 1.77 ± 0.06 | 1.87 ± 0.04 | 1.88 ± 0.05 | 0.207 | 0.966 |

Data are presented as the mean ± standard error of the mean. General linear model analysis was performed to test for associations between PTPN1 genotypes and each parameter after adjusting for age and gender. *q*-value: Benjamini–Hochberg procedure for secondary outcomes. BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment ratio; IRI, immunoreactive insulin; LDL, low-density lipoprotein; ln, log transformation.

**Table 3** | Association analysis of *PTPN1* tag single-nucleotide polymorphism of rs3787348 with changes of metabolic parameters at 3-month

| | rs3787348 | | | *P*-value | *q*-value |
|---|---|---|---|---|---|
| | G/G | G/T | T/T | | |
| ΔBMI (kg/m$^2$) | −1.9 ± 0.2 | −1.5 ± 0.1 | −1.2 ± 0.1 | 0.001 | – |
| ΔBodyweight (kg) | −5.1 ± 0.5 | −4.1 ± 0.2 | −3.1 ± 0.3 | 0.001 | 0.014* |
| ΔWaist circumference (cm) | −5.2 ± 0.7 | −4.1 ± 0.4 | −2.9 ± 0.4 | <0.001 | 0.013* |
| ΔSystolic blood pressure (mmHg) | −0.2 ± 7.7 | −3.2 ± 3.9 | −6.2 ± 4.8 | 0.562 | 0.787 |
| ΔDiastolic blood pressure (mmHg) | −4.6 ± 1.1 | −3.8 ± 0.6 | −3.1 ± 0.7 | 0.293 | 0.513 |
| ΔFasting plasma glucose (mmol/L) | −0.7 ± 0.2 | −0.5 ± 0.1 | −0.3 ± 0.1 | 0.096 | 0.336 |
| ΔHbA1c (%) | −0.3 ± 0.1 | −0.2 ± 0.1 | −0.1 ± 0.1 | 0.092 | 0.368 |
| ΔHbA1c (mmol/L) | −3.3 ± 1.1 | −2.2 ± 1.1 | −1.1 ± 1.1 | – | |
| ΔIRI (pmol/L) | −20 ± 21 | −16 ± 9 | −16 ± 13 | 0.825 | 0.924 |
| ΔHOMA-R | −1.8 ± 1.4 | −0.7 ± 0.5 | −1.7 ± 0.9 | 0.632 | 0.804 |
| ΔTotal cholesterol (mmol/L) | −0.4 ± 0.1 | −0.3 ± 0.1 | −0.2 ± 0.1 | 0.034 | 0.238 |
| ΔTriglyceride (mmol/L) | −0.6 ± 0.1 | −0.5 ± 0.1 | −0.4 ± 0.1 | 0.233 | 0.466 |
| ΔHDL cholesterol (mmol/L) | 0.02 ± 0.02 | 0.03 ± 0.01 | 0.04 ± 0.02 | 0.380 | 0.591 |
| ΔLDL cholesterol (mmol/L) | −0.3 ± 0.1 | −0.2 ± 0.1 | −0.1 ± 0.1 | 0.051 | 0.286 |
| ΔLeptin (ng/mL) | −3.9 ± 0.7 | −3.4 ± 0.8 | −1.1 ± 0.8 | 0.011 | 0.103 |
| ΔAdiponectin (μg/mL) | 0.1 ± 0.3 | 0.3 ± 0.2 | 0.1 ± 0.5 | 0.581 | 0.775 |

Data are presented as the mean ± standard error of the mean. General linear model analysis was performed to test for associations between *PTPN1* genotypes and changes of each parameter after adjusting for age, sex and baseline value of dependent variables. Changes from baseline conditions to those at 3 months were abbreviated as Δ. *q*-value: Benjamini–Hochberg procedure for secondary outcomes. *\**q*-value < 0.05. BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment ratio; IRI, immunoreactive insulin; LDL, low-density lipoprotein.

**Table 4** | Association analysis of *PTPN1* tag single-nucleotide polymorphism of rs6067484 with changes of metabolic parameters at 3 months

|  | rs6067484 | | | P-value | q-value |
|---|---|---|---|---|---|
|  | A/A | A/G | G/G | | |
| ΔBMI (kg/m$^2$) | −1.4 ± 0.1 | −1.3 ± 0.2 | −1.1 ± 0.4 | 0.398 | − |
| ΔBodyweight (kg) | −3.8 ± 0.2 | −3.3 ± 0.5 | −2.8 ± 1.0 | 0.351 | 0.578 |
| ΔWaist circumference (cm) | −4.0 ± 0.4 | −2.2 ± 0.7 | −0.5 ± 1.5 | 0.030 | 0.210 |
| ΔSystolic blood pressure (mmHg) | −3.7 ± 3.8 | −6.9 ± 7.7 | −10.1 ± 15.8 | 0.706 | 0.824 |
| ΔDiastolic blood pressure (mmHg) | −3.8 ± 0.5 | −2.5 ± 1.1 | −1.3 ± 2.3 | 0.292 | 0.545 |
| ΔFasting plasma glucose (mmol/L) | −0.5 ± 0.1 | −0.2 ± 0.2 | 0.0 ± 0.4 | 0.184 | 0.515 |
| ΔHbA1c (%) | −0.3 ± 0.1 | −0.4 ± 0.1 | −0.4 ± 0.2 | 0.513 | 0.718 |
| ΔHbA1c (mmol/L) | −3.2 ± 0.9 | −4.3 ± 1.1 | −4.2 ± 2.1 | − | |
| ΔIRI (pmol/L) | −18 ± 8 | −4 ± 15 | −10 ± 37 | 0.240 | 0.517 |
| ΔHOMA-R | −1.6 ± 0.5 | −0.5 ± 1.0 | −0.7 ± 3.9 | 0.150 | 0.467 |
| ΔTotal cholesterol (mmol/L) | −0.3 ± 0.1 | −0.3 ± 0.1 | −0.3 ± 0.2 | 0.869 | 0.936 |
| ΔTriglyceride (mmol/L) | −0.5 ± 0.1 | −0.4 ± 0.1 | −0.2 ± 0.2 | 0.198 | 0.504 |
| ΔHDL cholesterol (mmol/L) | 0.03 ± 0.01 | 0.05 ± 0.02 | 0.07 ± 0.05 | 0.452 | 0.666 |
| ΔLDL cholesterol (mmol/L) | −0.2 ± 0.1 | −0.2 ± 0.1 | −0.2 ± 0.1 | 0.903 | 0.936 |
| ΔLeptin (ng/mL) | −3.0 ± 0.5 | −3.3 ± 1.1 | −3.1 ± 1.8 | 0.989 | 0.989 |
| ΔAdiponectin (μg/mL) | 0.3 ± 0.2 | 0.1 ± 0.3 | 0.2 ± 1.0 | 0.281 | 0.562 |

Data are presented as the mean ± standard error of the mean. General linear model analysis was carried out to test for associations between *PTPN1* genotypes and changes of each parameter after adjusting for age, sex and baseline value of dependent variables. Changes from baseline conditions to those at 3 months were abbreviated as Δ. q-value: Benjamini–Hochberg procedure for secondary outcomes. BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment ratio; IRI, immunoreactive insulin; LDL, low-density lipoprotein.

rs3787348 and obesity. Additionally, the T allele of rs3787348 in *PTPN1* showed a tendency to be associated with higher HbA1c levels in the present study, although this relationship was not significant. One previous study showed a higher frequency of the G allele of the rs3787348 SNP in *PTPN1* in type 2 diabetes patients than in control individuals among white individuals[19]. The other study reported that insulin sensitivity was lower in the G allele of the rs3787348 SNP in Hispanic Americans[20]. A meta-analysis including 7,883 European individuals did not replicate these previous association[21], suggesting that these divergent findings imply different effects of variations of the *PTPN1* gene on the risk of obesity and type 2 diabetes in populations of different ethnic origins. These differences might be due to the difference in genetic architecture among individuals of different ethnic backgrounds. Accordingly, the significant association of rs3787348 with obesity might have a specific effect on Japanese individuals. Furthermore, previous studies only investigated the effects of individual SNPs that occurred at low frequencies (<2%) in the population, but in the present study, the minor allele (T allele) frequency of rs3787348 in *PTPN1* was relatively high (45.5%); therefore, the significant association between *PTPN1* rs3787348 and obesity might be noteworthy[21].

In the present study, we first showed that the T allele of rs3787348 in the *PTPN1* gene was significantly related to a lower reduction in BW, BMI and WC than the G allele of rs3787348; that is, the T allele was resistant to weight reduction therapy through diet and exercise guidance. Our results for

changes in TC and leptin associated with rs3787348 in the present study represented a nominal rather than a significant association. We carefully carried out weight reduction therapy in accordance with the Japan Society for the Study of Obesity guidelines,[25] and carefully assessed records of nutritional status, daily meals, daily steps and daily BW changes for each consultation in the outpatient clinic. To our knowledge, there have been several interventional studies on weight reduction therapy through metabolic surgery and low-energy diets[31,32], but there have been few observational longitudinal studies on weight reduction therapy through fundamental diet and exercise therapy based on guidelines. A longitudinal study reported that the melanocortin-4 receptor gene, which is a key protein regulating energy balance and adiposity, was associated with postpartum weight reduction and glycemic changes among women with prior gestational diabetes[33]. Mutations in the beta-3 adrenergic receptor were related to the response to lifestyle interventions in obese individuals and patients with impaired glucose tolerance in the Japan Diabetes Prevention Program[34,35]. The polymorphisms of several genes, such as the *UCP1* gene and genes of the renin–angiotensin system, were reported to be associated with the effects of a short-term diet intervention[32]. However, no studies have examined the relationship of the polymorphisms of *PTPNI* with response to weight reduction therapy; therefore, the present study is the first to show a significant relationship between the SNPs of *PTPN1* and the effects of weight reduction therapy. Because PTP1B is involved in insulin and leptin signaling, the T allele of rs3787348 in *PTPN1* might

cause further insulin resistance and leptin resistance, thus accumulating adipose tissue, which might make it difficult to lose bodyweight and serum leptin. Conversely, the G allele of rs3787348 in the *PTPN1* gene might not result in the accumulation of adipose tissue and thus is not resistant to weight reduction. In addition, the T allele of rs3787348 was associated with the smaller reduction of WC, which represents central obesity and metabolic syndrome. As PTP1B is involved in energy balance, and is an important signal for insulin and leptin, the present results are noteworthy for weight management and the prevention of CVD in obese patients.

A 3% reduction in weight is the minimum requirement to improve health hazards and obesity-related risk factors, such as hypertension, and glucose and lipid metabolism, in obese and overweight people in Japan[36]. The present study showed that 3–5% reductions of initial BW in all genotypes of the *PTPN1* SNP rs3787348 were observed. The G allele of rs3787348 in *PTPN1* had a tendency to show greater decreases in TC, LDL-C, FPG and HbA1c; conversely, the T allele of the SNP rs3787348 in *PTPN1* was resistant to decreases in cholesterol and glucose parameters. These differences of reductions in lipid and glucose profiles might be due to differences in weight reductions. In addition, because PTP1B is involved in insulin signaling, the larger decreases in lipid and glucose profiles in the G allele of rs3787348 in *PTPN1* might be due to directly enhanced insulin and leptin signaling by improving PTP1B action. Furthermore, the inactivation of *PTPN1* with antisense oligonucleotides regulates the expression of genes involved in lipogenesis, such as *SREBF1*, suggesting that PTP1B might play a role in lipid metabolism[14]. These detailed mechanisms for improving glycolipid metabolism through rs373847 mutations must be examined in the future. Several cross-sectional studies have shown that *PTPN1* SNPs are related to obesity and obesity-related complications, such as diabetes, dyslipidemia, hypertension, metabolic syndrome and coronary atherosclerosis[37–39]. Therefore, as *PTPN1* gene polymorphisms affected the degree of weight reduction and improvement of complications, such as glycolipid metabolism, in the present study, our results might contribute to future personalized medicine, such as more rigorous interventions with diet and exercise programs for obese patients with the T allele of rs3787348 in the *PTPN1* gene.

The present study had some limitations. The examination of *PTPN1* polymorphisms was limited to just two tagged SNPs. The two tagged SNPs that we examined (rs3787348 and rs6067484) do not cover the regions of exons 1, 9 and 10 of the 10 exons of *PTPN1*; accordingly, they do not cover the entire *PTPN1* gene region. In the future, SNPs that cover the entire *PTPN1* gene must be considered. The observation period during which weight reduction therapy was carried out was short. However, most obese individuals regain BW during long-term weight reduction treatment due to environmental and psychosocial factors[40]. Therefore, it might be appropriate to examine the degree of weight loss during a 3-month period, as in the present study. Although the records of daily meals,

steps and changes in BW were checked in the outpatient clinic, detailed energy balance and metabolism were not measured. Furthermore, a long-term prospective cohort study with a larger sample size must be carried out in individuals of various ethnicities to clarify the involvement of *PTPN1* polymorphisms and the clinical significance of the rs3787348 SNP in *PTPN1* for weight reduction therapy in obese patients. In the future, the potential functional implication of the SNP rs3787348 of the *PTP1B* gene must be elucidated.

In conclusion, the present study has provided the first evidence that a *PTPN1* polymorphism (rs3787348) was significantly associated with weight reduction and the improvement of glycolipid metabolism through weight reduction therapy in obese patients. These findings imply that the *PTPNI* polymorphism can be used as a clinical predictor of the efficacy of weight reduction and cardiovascular risks in obese patients. These results might help develop precision medicine for obese patients according to their genetic predisposition, which would lead to an improvement in the quality of medical care for the prevention of lifestyle diseases and CVD in a super-aging society.

## DISCLOSURE

The authors declare no conflict of interest.

## REFERENCES

1. Scherer PE, Hill JA. Obesity, diabetes, and cardiovascular diseases: a compendium. *Circ Res* 2016; 118: 1703–1705.
2. Douketis JD, Macie C, Thabane L, *et al*. Systematic review of long-term weight loss studies in obese adults: clinical significance and applicability to clinical practice. *Int J Obes (Lond)* 2005; 29: 1153–1167.
3. Iguchi A, Yamakage H, Tochiya M, *et al*. Effect of weight reduction therapy on obstructive sleep apnea syndrome and arterial stiffness in the patients with obesity and metabolic syndrome. *J Atheroscler Thromb* 2013; 20: 807–820.

4. Satoh N, Shimatsu A, Kato Y, *et al*. Evaluation of cardio-ankle vascular index, a new indicator of arterial stiffness independent of blood pressure, in obesity and metabolic syndrome. *Hypertens Res* 2008; 31: 1921–1930.

5. Satoh-Asahara N, Suganami T, Majima T, *et al*. Urinary cystatin C as a potential risk marker for cardiovascular disease and chronic kidney disease in patients with obesity and metabolic syndrome. *Clin J Am Soc Nephrol* 2011; 6: 265–273.

6. Satoh-Asahara N, Kotani K, Yamakage H, *et al*. Cardio-ankle vascular index predicts for the incidence of cardiovascular events in obese patients: a multicenter prospective cohort study (Japan Obesity and Metabolic Syndrome study: JOMS). *Atherosclerosis* 2015; 242: 461–468.

7. Hur Y-M, Kaprio J, Iacono WG, *et al*. Genetic influences on the difference in variability of height, weight and body mass index between Caucasian and east Asian adolescent twins. *Int J Obes (Lond)* 2008; 32: 1455–1467.

8. Locke AE, Kahali B, Berndt SI, *et al*. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015; 518: 197–206.

9. Akiyama M, Okada Y, Kanai M, *et al*. Genome-wide association study identifies 112 new loci for body mass index in the Japanese population. *Nat Genet* 2017; 49: 1458–1467.

10. Seely BL, Staubs PA, Reichart DR, *et al*. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. *Diabetes* 1996; 45: 1379–1385.

11. Lund IK, Hansen JA, Andersen HS, *et al*. Mechanism of protein tyrosine phosphatase 1B-mediated inhibition of leptin signaling. *J Mol Endocrinol* 2005; 34: 339–351.

12. Klaman LD, Boss O, Peroni OD, *et al*. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 2000; 20: 5479–5489.

13. Elchebly M, Payette P, Michaliszyn E, *et al*. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 1999; 283: 1544–1548.

14. Rondinone CM, Trevillyan JM, Clampit J, *et al*. Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. *Diabetes* 2002; 51: 2405–2411.

15. Ma Y-M, Tao R-Y, Liu Q, *et al*. PTP1B inhibitor improves both insulin resistance and lipid abnormalities in vivo and in vitro. *Mol Cell Biochem* 2011; 357: 65–72.

16. Digenio A, Pham NC, Watts LM, *et al*. Antisense inhibition of protein tyrosine phosphatase 1B with IONIS-PTP-1B$_{Rx}$ improves insulin sensitivity and reduces weight in overweight patients with type 2 diabetes. *Diabetes Care* 2018; 41: 807–814.

17. Bowden DW, Sale M, Howard TD, *et al*. Linkage of genetic markers on human chromosomes 20 and 12 to NIDDM in Caucasian sib pairs with a history of diabetic nephropathy. *Diabetes* 1997; 46: 882–886.

18. Dong C, Wang S, Li W-D, *et al*. Interacting genetic loci on chromosomes 20 and 10 influence extreme human obesity. *Am J Hum Genet* 2003; 72: 115–124.

19. Bento JL, Palmer ND, Mychaleckyj JC, *et al*. Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes. *Diabetes* 2004; 53: 3007–3012.

20. Palmer ND, Bento JL, Mychaleckyj JC, *et al*. Association of protein tyrosine phosphatase 1B gene polymorphisms with measures of glucose homeostasis in Hispanic Americans: the insulin resistance atherosclerosis study (IRAS) family study. *Diabetes* 2004; 53: 3013–3019.

21. Florez JC, Agapakis CM, Burtt NP, *et al*. Association testing of the protein tyrosine phosphatase 1B gene (PTPN1) with type 2 diabetes in 7,883 people. *Diabetes* 2005; 54: 1884–1891.

22. Kipfer-Coudreau S, Eberlé D, Sahbatou M, *et al*. Single nucleotide polymorphisms of protein tyrosine phosphatase 1B gene are associated with obesity in morbidly obese French subjects. *Diabetologia* 2004; 47: 1278–1284.

23. Cheyssac C, Lecoeur C, Dechaume A, *et al*. Analysis of common PTPN1 gene variants in type 2 diabetes, obesity, and associated phenotypes in the French population. *BMC Med Genet* 2006; 7: 44.

24. Traurig M, Hanson RL, Kobes S, *et al*. Protein tyrosine phosphatase 1B is not a major susceptibility gene for type 2 diabetes mellitus or obesity among Pima Indians. *Diabetologia* 2007; 50: 985–989.

25. Examination Committee of Criteria for 'Obesity Disease' in Japan; Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. *Circ J* 2002; 66: 987–992.

26. Spencer-Jones NJ, Wang X, Snieder H, *et al*. Protein tyrosine phosphatase-1B gene PTPN1: selection of tagging single nucleotide polymorphisms and association with body fat, insulin sensitivity, and the metabolic syndrome in a normal female population. *Diabetes* 2005; 54: 3296–3304.

27. Cauchi S, Meyre D, Dina C, *et al*. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 2006; 55: 2903–2908.

28. Teramoto T, Sasaki J, Ishibashi S, *et al*. Diagnosis of atherosclerosis. Executive Summary of the Japan Atherosclerosis Society (JAS) Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases in Japan-2012 Version. *J Atheroscler Thromb* 2014; 21: 296–298.

29. Hamada T, Kotani K, Nagai N, *et al*. Genetic polymorphisms of the renin-angiotensin system and obesity-related metabolic changes in response to low-energy diets in obese women. *Nutrition* 2011; 27: 34–39.

30. Cho H. Protein tyrosine phosphatase 1B (PTP1B) and obesity. *Vitam Horm* 2013; 91: 405–424.

31. Velázquez-Fernández D, Mercado-Celis G, Flores-Morales J, *et al*. Analysis of gene candidate SNP and ancestral origin associated to obesity and postoperative weight loss in a

cohort of obese patients undergoing RYGB. *Obes Surg* 2017; 27: 1481–1492.

32. Nagai N, Sakane N, Kotani K, *et al*. Uncoupling protein 1 gene -3826 A/G polymorphism is associated with weight loss on a short-term, controlled-energy diet in young women. *Nutr Res* 2011; 31: 255–261.

33. Carvalho AM, Shao P, Liu H, *et al*. The MC$_4$R genotype is associated with postpartum weight reduction and glycemic changes among women with prior gestational diabetes: longitudinal analysis. *Sci Rep* 2017; 7: 9654.

34. Yoshida T, Sakane N, Umekawa T, *et al*. Mutation of Beta 3-adrenergic-receptor gene and response to treatment of obesity. *Lancet* 1995; 346: 1433–1434.

35. Sakane N, Sato J, Tsushita K, *et al*. Effects of lifestyle intervention on weight and metabolic parameters in patients with impaired glucose tolerance related to beta-3 adrenergic receptor gene polymorphism Trp64Arg(C/T): results from the japan diabetes prevention program. *J Diabetes Investig* 2016; 7: 338–342.

36. Muramoto A, Matsushita M, Kato A, *et al*. Three percent weight reduction is the minimum requirement to improve health hazards in obese and overweight people in Japan. *Obes Res Clin Pract* 2014; 8: e466–e475.

37. El-Halim EFA, Khodair SZ, Kora MA, *et al*. Study of clinical significance of protein tyrosine phosphatase-1B gene polymorphism in essential hypertension and dyslipidemia. *Menoufia Med J* 2016; 29: 160–166.

38. Olivier M, Hsiung CA, Chuang L-M, *et al*. Single nucleotide polymorphisms in protein tyrosine phosphatase 1beta (PTPN1) are associated with essential hypertension and obesity. *Hum Mol Genet* 2004; 13: 1885–1892.

39. Burdon KP, Bento JL, Langefeld CD, *et al*. Association of protein tyrosine phosphatase-N1 polymorphisms with coronary calcified plaque in the diabetes heart study. *Diabetes* 2006; 55: 651–658.

40. MacLean PS, Higgins JA, Giles ED, *et al*. The role for adipose tissue in weight regain after weight loss. *Obes Rev* 2015; 16 (Suppl 1): 45–54.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1** | Association analysis of *PTPN1* tag single-nucleotide polymorphism of rs6067484 with metabolic parameters.
**Appendix S1** | STROBE Statement – checklist of items that should be included in reports of observational studies.