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Response to the dipeptidyl peptidase-4 inhibitors in Japanese patients with type 2 diabetes might be associated with a diplotype of two single nucleotide polymorphisms on the interleukin-6 promoter region under a certain level of physical activity

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ABSTRACT

Aims/Introduction: Muscle-derived interleukin-6 (IL-6) has been reported to promote glucagon-like peptide-1 (GLP-1) secretion, and we explored the association of single nucleotide polymorphisms (SNPs) in the human IL-6 promoter region with the responsiveness to dipeptidyl peptidase-4 inhibitors (DPP-4Is), drugs that increase circulating GLP-1.

Materials and Methods: The present observational study enrolled Japanese patients with type 2 diabetes who took a DPP-4I over 3 months, and most of the clinical information was collected retrospectively. We defined non-responders as those having less than a 0.2% decrease of the glycated hemoglobin level at 3 or 4 months after starting DPP-4I treatment. Physical activity was retrospectively estimated by the Japanese short version of International Physical Activity Questionnaire.

Results: We studied 316 patients whose physical activity corresponding to the season of the DPP-4I administration was estimated. The non-responder rate was 29.7%. We analyzed rs1800796 and rs2097677, both are suggested to be functional in Japanese. Multivariate analysis for all patients showed that the adjusted odds ratio for the non-responder risk of the diplotype rs1800796 G/*–rs2097677 A/* against C/C-G/G (OR_G*A*) was 0.445 ($P = 0.068$). When patients were stratified by the International Physical Activity Questionnaire into low ($n = 149$) and moderate/high ($n = 167$) activity groups, however, OR_G*A* in each group was 1.58 ($P = 0.615$) and 0.153 ($P = 0.003$), respectively.

Conclusions: The diplotype rs1800796 G/*–rs2097677 A/* might contribute to responsiveness to DPP-4Is in Japanese patients with type 2 diabetes under a certain level of physical activity. However, further investigation is warranted to confirm this.

INTRODUCTION

Recently, dipeptidyl peptidase-4 (DPP-4) inhibitors (DPP-4Is) have increasingly been used for patients with type 2 diabetes worldwide. This class of antidiabetic drugs specifically inhibits

DPP-4, which breaks down glucagon-like peptide (GLP)-1 and gastric inhibitory peptide (GIP). Consequently, DPP-4Is increase plasma levels of these two gut hormones. GLP-1 and GIP stimulate insulin secretion in a blood glucose level-dependent manner, and GLP-1 has the ability to inhibit glucagon secretion. These peptides are thus expected to improve the outcomes of the treatment for patients with type 2 diabetes, as there is little risk of hypoglycemia or weight gain, and both are suggested to exert a protective effect on pancreatic β-cells[1]. Interestingly, a recent meta-analysis showed that DPP-4Is decrease glycated hemoglobin (HbA1c) levels more markedly in Asians than in non-Asians[2], and clinical factors for unresponsiveness to DPP-4Is have in fact been more intensively studied in Asian subjects[3–9]. However, no genetic factors are known to be responsible for the efficacy of DPP-4Is.

To explore the role of genetic factors in unresponsiveness to DPP-4Is, we focused on a recent report stating that in animal models, interleukin-6 (IL-6) derived from muscle cells during exercise enhances GLP-1 synthesis and secretion by intestinal L cells, and also affects pancreatic α-cell properties[10]. Although this apparently novel cytokine network in patients with type 2 diabetes has yet to be elucidated, human studies suggest that physical training could enhance insulin secretory capacity in type 2 diabetes[11], and postprandial exercise acutely improved GLP-1 secretion in non-obese healthy subjects[12]. If systemically elevated IL-6 works similarly in humans, genetic variants that are known to enhance the transcription of IL-6 might improve GLP-1 synthesis and secretion. A harmonious relationship between the exercise-induced increase in GLP-1 and inhibition of DPP-4 throughout the day would result in a sustained elevation of circulating GLP-1. Herein, we hypothesized that single nucleotide polymorphisms (SNPs) in the IL-6 promoter region might be associated with the efficacy of DPP-4Is, and explored this possible association in Japanese patients with type 2 diabetes.

MATERIALS AND METHODS

Study Participants
We carried out an observational study of Japanese patients with type 2 diabetes who visited the Division of Diabetes and Metabolism, Department of Internal Medicine, Iwate Medical University, Morioka, Japan, between 1 December 2009 and 31 March 2013. We screened all outpatients at our department who had taken one of the available DPP-4 inhibitors for the first time ($n = 426$). Exclusion criteria were: (i) hospital admission within half a year of starting a DPP-4I; (ii) moderate to severe liver disease; (iii) renal failure; (iv) steroid therapy; (v) recent resumption of antidiabetic medication; and (vi) any type of ongoing anticancer therapy. Ultimately, 331 eligible patients participated in the present study. We obtained written informed consent from all of the participants according to the Declaration of Helsinki. The present study was approved by the institutional ethics committee of Iwate Medical University.

Diagnostic Criteria
The diagnosis of type 2 diabetes mellitus was confirmed by specialists in our department. Hypertension was defined as having at least one of the following: (i) systolic blood pressure 140 mmHg or more; (ii) diastolic blood pressure 90 mmHg or more; and/or (iii) current medication. Likewise, dyslipidemia was defined as having at least one of the following: (i) low-density lipoprotein cholesterol 140 mg/dL (3.1 mmol/L) or more; (ii) fasting triglyceride 150 mg/dL (1.69 mmol/l) or more; (iii) high-density lipoprotein-cholesterol <40 mg/dL (1.03 mmol/L), and/or (iv) current medication. At baseline, blood samples were obtained *ad libitum*. Lipid counts at baseline were missing in one patient, and data at 1 month before the start of a DPP-4I were used to evaluate dyslipidemia in this patient. Obesity was defined as body mass index (BMI) ≥25 kg/m^2 according to the Japan Association for the Study of Obesity.

Management of Diabetes
Diabetes was managed by the patient's physician, and the use of any other type of antidiabetic medication(s) together with a DPP-4I was allowed so long as it was covered by the public insurance system of Japan. It should be noted that when a DPP-4I was given as an add-on therapy with sulfonylurea (SU), the dose of sulfonylurea was reduced according to the recommendation of the Japan Diabetes Society (JDS), because of an unexpectedly high incidence of severe hypoglycemia[5]. However, such a dose reduction reportedly does not diminish efficacy[5]. The interval of the hospital visits was also determined by physicians, and patients visited our hospital every 1, 2 or 3 months.

Data Collection and the Outcome Definition
As aforementioned, patients were recruited at least 3 months after starting treatment with a DPP-4I, and the laboratory data, medical history and anthropometric data before recruitment were retrospectively obtained from medical records. BMI was computed from the measurement of height at the first visit to our hospital and the self-reported weight at every visit to our hospital department. The HbA1c value (%) was converted to the National Glycohemoglobin Standardization Program equivalent by the JDS formula[13]. The outcome was the HbA1c reduction at 3 months, and if that value was not available, the 4-month HbA1c value was used instead (17.4% of participants). Then, non-responders were defined as those who had less than a 0.2% decrease in the outcome HbA1c value, which was a little more stringent than the definition in some preceding studies[3,5]. Serum C-peptide, serum high-sensitivity C-reactive protein (hsCRP), plasma glucagon and plasma IL-6 at rest were examined on the day of recruitment or at the first visit after recruitment, and therefore only the post-treatment data were available for these counts.

Estimation of Physical Activity by Questionnaire
Because of the study design, we could not ascertain physical activity during the observational period. Instead, we computed

an estimation of physical activity at the start of a DPP-4I as follows. We gave patients a short Japanese version of the International Physical Activity Questionnaire (IPAQ) twice[14]; the first was from 1 November 2012 to 31 May 2013 (winter-to-spring session) and the second from 1 July 2013 to 30 September 2013 (summer-to-autumn session). The former IPAQ was carried out at the time of recruitment. Total metabolic equivalents (METs)-min/week were computed according to the literature[15] by multiplying the intensity of the activity and the corresponding duration in a week. All patients provided their activities during the winter-to-spring session, but 37 of the 331 patients moved to other hospitals, and we were thus unable to obtain summer-to-autumn session data from them. We decided to use either of the IPAQ data according to the season in which each patient had started the DPP-4I, and consequently 316 out of 331 patients were able to be analyzed.

Identification of SNPs in the IL-6 Promoter Region
Peripheral blood samples were once frozen in special sample tubes, stored in a freezer and the bulk of the anonymous samples were sent to an external laboratory for genotyping (BEX Co. Ltd., Tokyo, Japan). Each SNP was identified by the Q-probe method.

Statistical Analysis
Data are shown as numbers with percentages or means ± standard deviation, or as medians with an interquartile range (IQR; 25–75%). Continuous variables were compared by the unpaired t-test, Mann–Whitney U-test or Kruskal–Wallis test where appropriate. The categorical variables were compared by Fisher's exact test or the χ^2-test. Then, binary logistic regression analysis was carried out to detect the predictors for non-responders to DPP-4Is. Haplotype estimation was carried out using Arlequin 3.11[16]. Statistical analyses were carried out using SPSS 17.0 (IBM Japan Ltd, Tokyo, Japan).

RESULTS
We studied a total of 316 Japanese patients with type 2 diabetes, and 94 (29.7%) were defined as non-responders to DPP-4Is. Baseline characteristics are shown in Table 1, and information on DPP-4Is and other classes of antidiabetic medication are in Table 2. The proportion of four DPP-4Is prescribed to the responders and non-responders was not significantly different. In the univariate analysis, the baseline HbA1c level was significantly higher in responders than in non-responders, and also the duration since being diagnosed with diabetes was longer in the responders (Table 1). Almost no change in BMI was observed in either responders or non-responders during DPP-4I treatment (data not shown). The plasma IL-6 level at rest after 3 or more months of treatment was 1.8 ng/mL (IQR 1.2–3.0) in responders and 2.2 ng/mL (IQR 1.5–3.1) in non-responders ($P = 0.091$). The hsCRP level was 0.054 mg/dL (IQR 0.026–0.149) in responders and 0.083 mg/dL (0.028–0.175) in non-responders ($P = 0.308$). hsCRP

Table 1 | Characteristics of patients according to responsiveness and non-responsiveness to dipeptidyl peptidase-4 inhibitors

Variables	Responders (n = 222)	Non-responders (n = 94)	P
Sex, men (%)	143 (64.4)	65 (69.1)	0.496
Age (years)	62.9 ± 11.8	62.9 ± 11.5	0.980
Body mass index (kg/m²)	24.6 ± 3.97	24.4 ± 4.69	0.610
Duration of diabetes (years)	12.2 ± 8.77	9.46 ± 7.92	0.010
Hypertension, n (%)	155 (69.8)	59 (62.7)	0.274
Antihypertensive medication, n (%)	142 (64.0)	53 (56.4)	0.205
Dyslipidemia, n (%)	151 (68.0)	63 (67.0)	0.967
Antidyslipidemic medication, n (%)	134 (60.4)	58 (61.7)	0.823
Family history of diabetes, n (%)	134 (60.4)	57 (60.6)	>0.999
Smoking (never/ex/current), n (%)	115/59/48 (51.8/26.6/21.6)	44/33/17 (46.8/35.1/18.1)	0.304
Habitual drinking, n (%)	122 (55.0)	66 (70.2)	0.016
Casual plasma glucose (mmol/L)	9.40 ± 2.98	8.76 ± 3.26	0.091
Baseline HbA1c (%) (IFCC, mmol/mol)	7.72 ± 0.99 60.9 ± 10.8	7.23 ± 1.35 55.5 ± 14.8	<0.001
Change in HbA1c levels from the baseline	−0.80 ± 0.47	0.28 ± 0.47	<0.001
LDL cholesterol (mmol/L)	2.62 ± 0.71*	2.58 ± 0.65	0.647
HDL cholesterol (mmol/L)	1.52 ± 0.39*	1.53 ± 0.41	0.793
METs – min/week, median (IQR)			
Winter-to-spring session (n = 316)	785 (198–2374)	767 (480–1880)	0.637
Summer-to-autumn session (n = 294)	990 (297–2822)†	900 (308–2538)‡	0.801
Corresponding to the start of DPP-4Is	870 (252–2420)	836 (484–1734)	0.784

Data are numbers with percentages, mean ± standard deviation or the median with interquartile range (IQR). Physical activity expressed as metabolic equivalents (METs) min/week was estimated in two seasonal sessions, and either of the two International Physical Activity Questionnaires was used for estimation corresponding to the start of dipeptidyl peptidase-4 inhibitors (DPP-4Is). *Values from one patient are missing. †Values from 17 patients are missing. ‡Values from five patients are missing. HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

correlated significantly with BMI levels in the present study (Spearman's correlation coefficient [SC] 0.301, $P < 0.001$)[17], but IL-6 did not, although hsCRP and IL-6 levels correlated significantly with each other (SC 0.553, $P < 0.001$). Lower physical activity correlates with higher circulating IL-6 at rest[18], and in the present study the levels of plasma IL-6 at rest measured in the winter-to-spring session showed a significant negative

Table 2 | Medication for diabetes in responders and non-responders

Variables	Responders (n = 222)	Non-responders (n = 94)	P
Administered DPP-4Is (n)			0.526
Sitagliptin	164	65	
Vildagliptin	21	15	
Alogliptin	34	13	
Linagliptin	2	1	
Teneligliptin	1	0	
Way of the administration of DPP-4Is (n)			<0.001
First-time monotherapy	34	4	
Switch from an OHA	36	35	
Add-on	104	31	
Add-on with a dose-reduction of an SU	27	7	
Add-on with a dose-reduction of another medication (except SU)	21	17	
Additional medications at the start, n (%)			
None	45 (20.3)	24 (25.5)	0.301
Insulin	55 (24.8)	29 (30.9)	0.328
Sulfonylurea	79 (35.6)	30 (31.9)	0.618
Metformin	101 (45.4)	23 (24.5)	<0.001
α-Glucosidase inhibitor	59 (26.6)	27 (28.7)	0.800
Thiazolidinedione	30 (13.5)	10 (10.6)	0.605

Data are number or number (%). DPP-4Is, dipeptidyl peptidase-4 inhibitors; OHA, oral hypoglycemic agent; SU, sulfonylurea.

correlation with METs-min/week in that session (SC –0.206, $P < 0.001$). In patients who completed IPAQ twice ($n = 294$), the two estimates of physical activity had a significant correlation (SC 0.313, $P < 0.001$).

Effects of Individual IL-6 SNPs on the Risk of Being a Non-Responder

Rs1800795 has been implicated in immunological and metabolic abnormalities[19], but all patients in the present study had only the G allele, which has been confirmed in subjects of Japanese ethnicity[20]. The rs1800796 G allele was reported to be associated with higher plasma IL-6 levels in normal Han Chinese[21] and Asian patients with type 2 diabetes[22,23], whereas it was associated with a lower level of IL-6 in Hong Kong Chinese[24]. The rs2097677 A allele was identified in a large cohort of Japanese using CRP levels as a biomarker[25], although this has not been replicated in other ethnicities[26], and its effect on patients with type 2 diabetes is unknown. Herein, we analyzed the latter two SNPs.

First, we tested the single locus effect of rs1800796 or rs2097677 on the risk of non-responsiveness to DPP-4Is. There was no significant difference in non-responder rates between the rs1800796 and rs2097677 genotypes (Table 3, upper). Next, we analyzed the effects of diplotypes composed of the two loci, but there was no significant difference in non-responder rates between different diplotypes (Table 3, lower). Multiple SNPs on a promoter region might have a cis-effect on regulation of the gene transcription[27,28]. Then we computed estimations of haplotype frequencies[16], but found no significant difference between responders and non-responders (Appendix S1). There were no significant differences in plasma IL-6 or hsCRP levels under DPP-4I treatment among different genotypes or diplotypes of these SNPs (data not shown).

Effect of Diplotypes of the Two SNPs on Non-Responders

The analysis described here failed to identify any significant effect of SNPs *per se*, but the assumed source of IL-6 in the present study was muscle, and physical activity might exert an effect on unresponsiveness to DPP-4Is. In addition, adipose tissue is known to be responsible for chronic elevation

Table 3 | Summary of the genotypes and diplotypes of the two single nucleotide polymorphism loci in the interleukin-6 promoter region

SNP	Genotype	Responders (n)	Non-responders (n)	Non-responder rates	P
Genotypes of each locus					
rs1800796	C/C	141	66	0.319	0.494
	G/C	74	25	0.253	
	G/G	7	3	0.300	
rs2097677	G/G	139	66	0.322	0.244
	A/G	77	24	0.278	
	A/A	6	4	0.400	

rs1800796	rs2097677	Responders (n)	Non-responders (n)	Non-responder rates	P
Diplotypes of the two SNP loci					
C/C	G/G	98	50	0.339	0.469
C/C	A/G or A/A	43	16	0.271	
G/C or G/G	G/G	41	16	0.281	
G/C or G/G	A/G or A/A	40	12	0.231	

Data are presented as numbers or rates. SNP, single nucleotide polymorphism.

of circulating IL-6, although the plasma IL-6 level at rest and BMI had no significant correlation in the present study. Also, clinical outcome would presumably be a composite effect of the two SNPs rather than a single locus effect. Here, it would be rational to evaluate the role of IL-6 SNPs on the basis of the diplotypes of the two SNPs, physical activity and BMI levels.

Before the multivariate analysis, we analyzed the plasma IL-6 levels in the four diplotypes composed of the two SNPs between groups having different physical activity levels. According to the literature, the duration and intensity of exercise are important factors for substantially raising the circulating IL-6 level in humans, and as such the sum of short bouts of low-intensity exercise might not lead to an increase in the circulating IL-6 level sufficient to exert a meaningful systemic effect[29]. To better reflect the intensity of exercise, we used a generalized and intensity-based categorization (low, moderate and high)[15]. It should be noted that only the IL-6 levels at rest in the winter-to-spring session were measured, and the measures are presumed not to directly correlate with the exercise-induced IL-6 secretion. Approximately half of the patients belonged to the low categorization ($n = 147$), whereas the moderate and high categorizations were smaller ($n = 69$ and 100, respectively). Thus, we divided patients into two groups, the low(w) and moderate/high(w) groups, by the winter-to-spring session IPAQ (thus "[w]" represents the winter session), and compared the plasma IL-6 levels between four diplotypes (Table 4). The result was that IL-6 levels at rest were not significantly different between the eight groups ($P = 0.193$; Table 4). We also analyzed the difference in the levels of hsCRP, BMI or other laboratory data in the eight groups, but found no significant difference (data not shown).

Table 4 | Plasma interleukin-6 levels at rest in the winter-to-spring session, physical activity in that session, and the diplotypes of interleukin-6 single nucleotide polymorphisms

Physical activity	Diplotype	Plasma IL-6 levels at rest (ng/mL)	n
Low(w) ($n = 147$)	C/C-G/G	2.1 (1.3–3.2)	67
	C/C-A/*	1.9 (1.6–3.2)	32
	G/*-G/G	2.1 (1.6–3.6)	29
	G/*-A/*	2.0 (1.3–4.5)	19
Moderate/high(w) ($n = 169$)	C/C-G/G	1.6 (1.1–2.8)	81
	C/C-A/*	2.1 (1.0–3.5)	27
	G/*-G/G	1.7 (1.1–2.6)	28
	G/*-A/*	1.7 (1.0–2.8)	33

Data are expressed as median (interquartile range 25–75%), since plasma interleukin (IL)-6 levels at rest were not normally distributed. International Physical Activity Questionnaire data used are those in the winter-to-spring session when plasma IL-6 was measured, and the patients are grouped into low(w) and moderate/high(w) by the intensity-based categorization ("[w]" represents the winter session). The difference in the average of plasma IL-6 levels between eight groups was not significant ($P = 0.193$ by Kruskal–Wallis test).

Next, we carried out a main multivariate analysis for all patients to evaluate the effect of the diplotype of the two SNPs on non-responder risk, using a logistic regression analysis with adjustment for sex, age, duration of being diagnosed with diabetes, BMI, physical activity, family history of diabetes, smoking, habitual drinking, hypertension, dyslipidemia, the HbA1c level at the start of DPP-4I treatments, classes of other antidiabetic medications and the change of antidiabetic medications at the start of DPP-4Is. The diplotype rs1800796 G/*–rs2097677 A/* had carried a marginal risk reduction for non-responders as compared with the C/C-G/G group (odds ratio 0.445, 95% confidence interval [CI] 0.187–1.06, $P = 0.068$; Table 5). Duration of diabetes and use of metformin were negatively associated with non-responder risk, whereas habitual drinking was a risk for being non-responders. Any change of the antidiabetic medications was included in the present study, and the switch from an oral hypoglycemic agent (OHA) was a strong risk factor for non-responders. Also, add-on of a DPP-4I to other medication(s) was a risk factor, except for the case with a dose-reduction of a SU[5].

Finally, we divided patients into two groups (the low group [$n = 149$] and the moderate [$n = 87$]/high [$n = 80$] group) by the IPAQ data corresponding to the season in which DPP-4 inhibitors had been administered (therefore the groups were different from those in Table 4). Then we carried out similar logistic regression analysis for the two physical activity subgroups separately. It should be noted that the patients who had taken DPP-4Is as a first-time monotherapy were all responders in the low group, and the value of the term representing the change of antidiabetic medication at the start of DPP-4Is was modified to construct a valid and comparable regression model. That is, we combined the patients taking a first-time monotherapy of DPP-4Is and the patients taking an add-on therapy of DPP-4Is with a dose-reduction of a SU. This was based on the analysis for all patients in which the two categories had no significant difference in the risk for being non-responders (Table 5). The result showed that the diplotype rs1800796 G/*–rs2097677 A/* had a lower risk for being non-responders than C/C-G/G in the moderate/high group (adjusted odds ratio 0.153, 95% CI 0.044–0.535, $P = 0.003$), but not in the low group (Table 6). In the low group, duration of diabetes was negatively associated with the non-responder risk, whereas BMI and the switch from an OHA were positively associated with it. Also, use of insulin, SU and α-glucosidase inhibitors had a positive association with the non-responder risk, and current smoking had a negative association with it. In the moderate/high group, use of metformin was negatively associated with the non-responder risk, and switch from an OHA and add-on of a DPP-4I to other medication(s) were positively associated with it. Habitual drinking had a positive association with the non-responder risk in the moderate/high group.

DISCUSSION

The present study suggests that a combination of two SNPs in the IL-6 promoter region can reduce the risk of being a

Table 5 | Binary logistic regression analysis to detect predictors for non-responders

Variables	Odds ratios for non-responders	P
Diplotypes of SNPs		
C/C-G/G	Referent	
C/C-A/*	0.682 (0.322–1.45)	0.318
G/*-G/G	0.782 (0.361–1.69)	0.532
G/*-A/*	0.445 (0.187–1.06)	0.068
Sex (men)	0.720 (0.336–1.54)	0.398
Age (years)	0.998 (0.969–1.03)	0.892
Duration of diabetes (years)	0.956 (0.918–0.994)	0.025
BMI (kg/m^2)	1.06 (0.976–1.15)	0.164
Habitual drinking (yes)	1.94 (1.01–3.73)	0.048
Smoking		
Never	Referent	
Ex	0.941 (0.444–1.99)	0.873
Current	0.568 (0.243–1.33)	0.192
Family history of diabetes	1.20 (0.654–2.19)	0.560
Hypertension	0.568 (0.308–1.049)	0.071
Dyslipidemia	1.21 (0.64–2.25)	0.559
HbA1c levels at the start (%)	0.758 (0.546–1.05)	0.096
METs (1,000 min/week)	0.964 (0.891–1.04)	0.356
Other medications		
Insulin	1.60 (0.631–4.03)	0.323
Sulfonylurea	1.66 (0.726–3.81)	0.229
Metformin	0.310 (0.154–0.623)	<0.001
α-Glucosidase inhibitors	1.36 (0.711–2.60)	0.353
Thiazolidinedione	0.889 (0.351–2.25)	0.804
Ways of the administration of DPP-4Is		
First-time monotherapy	Referent	
Switch from an oral hypoglycemic agent	11.0 (3.08–39.1)	<0.001
Add-on to other medication(s)	3.93 (1.035–14.9)	0.044
Add-on with a dose-reduction of an SU	2.58 (0.491–13.6)	0.263
Add-on with a dose-reduction of another medication (except SU)	7.00 (1.41–34.6)	0.017

Data are presented as adjusted odds ratios with 95% confidence intervals. Diplotypes of the two single nucleotide polymorphisms (SNPs) are shown in the order rs1800796–rs2097677. The glycated hemoglobin (HbA1c) value is shown as a percentage. Odds ratio for total metabolic equivalents (METs)-min/week is per 1,000 increase. Otherwise, odds ratios for continuous variables are per one increase. BMI, body mass index; DPP-4Is, dipeptidyl peptidase-4 inhibitors; SU, sulfonylurea.

non-responder to DPP-4Is in Japanese patients, but this benefit requires a certain level of physical activity. In the report by Ellingsgaard *et al.*,[10] not only injection of a high dose of IL-6 that mimicked the acute increase in IL-6 produced by a long bout of vigorous exercise, but also a low dose of IL-6 that mimicked the mild chronic increase in IL-6 produced by a high-fat diet led to an increase in circulating GLP-1 in mice. That is the reason we first hypothesized that IL-6 SNPs might contribute to the enhancement of GLP-1 secretion from muscle

as well as adipose tissue in response to IL-6. The present study result did not suggest the role of adiposity in the possible effect of the SNPs on the efficacy of DPP-4Is.

The role of circulating IL-6 in glucose metabolism is a debated subject. Plasma IL-6 is increased in obesity and type 2 diabetes, and a nested case–control study from a large-scale cohort showed that elevated IL-6 is a risk factor for the development of type 2 diabetes[30]. However, studies in IL-6 knockout mice showed a role of IL-6 in the brain that counteracts obesity[31], and a recent study reported beneficial effects of IL-6 secreted from muscle on the improvement of glucose uptake and fat oxidation[32]. Furthermore, macrophage-derived IL-6 has been implicated in the beneficial action of adiponectin on insulin sensitivity in the murine liver[33]. Thus, various forms of cell-derived IL-6 reportedly play beneficial roles in glucose and fat metabolism.

The present study suggests, but does not show, a beneficial role of IL-6 in the efficacy of a class of medication for type 2 diabetes. The precise mechanism by which these SNPs reduce the likelihood of being a non-responder to DPP-4Is remains to be clarified. There is little information on the regulatory mechanism of these two SNPs in humans. An *in vitro* study suggested haplotype- and cell type-dependent transcriptional regulation of the IL-6 promoter region[28]. Furthermore, a recent report suggested that very specific *in vivo* conditions are required to elucidate the roles of SNPs in the IL-6 promoter region[26], which highlights the important role of epigenetics under various environmental conditions. In fact, reports on the effects of the rs1800796 G allele have not been consistent to date[21–24]. Thus, it is crucial to show differences in exercise-dependent increases in IL-6 or GLP-1 between different genotypes in humans, but this was beyond the scope of the present study. Further investigation is required for direct evidence on this issue.

Another limitation in the present study was that we could not rule out the possibility that DPP-4Is affect IL-6 production in several tissues. DPP-4 is an enzyme that degrades a lot of substrates, and recently a reduced production of IL-6 in monocytes as a result of DPP-4 inhibition has been reported[34]. Although the effects of DPP-4Is on IL-6 synthesis in or secretion by muscle are not known, the DPP-4 expression level in muscle is low[35], and we speculate that DPP-4Is are unlikely to exert a substantial effect on muscle-derived IL-6. However, a recent study showed that DPP-4 is secreted from adipose tissue, and that it is a biomarker for metabolic syndrome[36]. We have no information as to whether or not the secretion of DPP-4 in this tissue contributes to the regulation of IL-6 secretion in adipose tissue. Furthermore, muscle cells from patients with type 2 diabetes have a defective response to IL-6[37], suggesting that resistance to IL-6 might exist in some tissues of patients with type 2 diabetes. Further investigation is warranted to show the link between circulating IL-6 and GLP-1 secretion specifically in patients with type 2 diabetes under the administration of DPP-4Is.

Covariates for affecting the non-responder risk were identified in the main and subgroup analyses, although our primary

Table 6 | Binary logistic regression analysis to detect predictors for non-responders in the patients stratified by physical activity

Physical activity groups	Low (n = 149)		Moderate or high (n = 167)	
Variables	Odds ratio	P	Odds ratio	P
Diplotypes				
C/C-G/G	Referent		Referent	
C/C-A/*	1.48 (0.436–5.00)	0.531	0.411 (0.134–1.27)	0.121
G/*-G/G	1.60 (0.416–6.14)	0.494	0.726 (0.238–2.21)	0.573
G/*-A/*	1.58 (0.265–9.44)	0.615	0.153 (0.044–0.535)	0.003
Sex (men)	1.31 (0.312–5.48)	0.715	0.449 (0.150–1.34)	0.151
Age (years)	0.975 (0.919–1.03)	0.402	1.03 (0.983–1.07)	0.242
Duration of diabetes	0.884 (0.813–0.960)	0.003	0.980 (0.929–1.03)	0.447
BMI (kg/m^2)	1.23 (1.06–1.43)	0.007	0.979 (0.862–1.11)	0.739
Habitual drinking (yes)	1.23 (0.345–4.39)	0.749	2.96 (1.09–8.06)	0.033
Smoking				
Never	Referent		Referent	
Ex	0.352 (0.086–1.43)	0.144	1.56 (0.515–4.70)	0.433
Current	0.179 (0.032–0.984)	0.048	0.451 (0.132–1.54)	0.204
Family history of diabetes	0.730 (0.237–2.25)	0.583	1.63 (0.672–3.97)	0.279
Hypertension	0.334 (0.102–1.09)	0.070	0.597 (0.250–1.42)	0.244
Dyslipidemia	0.800 (0.261–2.45)	0.696	1.424 (0.574–3.53)	0.446
HbA1c levels at the start (%)	0.763 (0.442–1.32)	0.331	0.876 (0.540–1.42)	0.593
METs (1,000 min/week)	0.758 (0.304–1.90)	0.554	0.938 (0.842–1.04)	0.241
Other medications				
Insulin	10.2 (1.64–63.6)	0.013	0.868 (0.242–3.11)	0.827
Sulfonylurea	6.42 (1.27–32.5)	0.025	1.36 (0.495–3.73)	0.553
Metformin	0.396 (0.119–1.31)	0.130	0.247 (0.091–0.667)	0.006
α-Glucosidase inhibitor	4.03 (1.08–15.1)	0.039	0.801 (0.297–2.16)	0.661
Thiazolidinedione	0.672 (0.113–3.99)	0.662	1.92 (0.530–6.97)	0.321
Ways of the administration of DPP-4Is				
First-time monotherapy or add-on with a dose-reduction of an SU†	Referent		Referent	
Switch from an oral hypoglycemic agent	33.6 (5.57–202)	<0.001	7.32 (1.87–28.6)	0.004
Add-on to other medication(s)	0.610 (0.113–3.29)	0.566	5.68 (1.63–19.7)	0.006
Add-on with a dose-reduction of another medication (except SU)	2.76 (0.332–22.9)	0.348	9.05 (1.42–57.6)	0.020

Data are adjusted odds ratio with 95% confidence intervals for the non-responder risk. BMI, body mass index; DPP-4Is, dipeptidyl peptidase-4 inhibitors; METs, total metabolic equivalents. †The patients with first-time monotherapy and the patients with add-on therapy with a dose-reduction of a sulfonylurea (SU) were combined into one referent group in order to construct a valid logistic regression model.

outcome is the association between IL-6 SNPs and the non-responder risk, and those covariates are not the primary outcome in the present study. We as yet have no plausible explanations except for the beneficial effect of metformin[38]. In the present study, use of α-glucosidase inhibitors was a risk factor for being a non-responder only in the low physical activity group, but miglitol was reported to enhance GLP-1 secretion[39]. Use of insulin and SU were also risk factors only in the low group, and we speculate that patients using these medications might have had a lower insulin secretory capacity. Doses and classes of antidiabetic medications were not controlled in the present study, and we cannot clarify the effects of the add-on therapy. Also, the longer the duration of since being diagnosed

with diabetes the lower the risk for being a non-responder tended to be, and this is also inconsistent with the results described in a preceding report[5]. Here, we cannot exclude information bias on the duration of diabetes. Drinking might be associated with a higher caloric intake, and is likely to increase the non-responder risk. BMI was a risk factor only in the low group, which might be the result of obesity- and lower physical activity-associated insulin resistance. Also, current smoking, which is known to enhance energy expenditure, seemed to have an association in the low group.

The present study had limitations other than those already described. First, we observed the effect of the SNPs on the non-responder risk only in the subgroup analysis, not in the

main analysis. Herein, the problem of multiple testing might exist. Second, this was an observational study in which many DPP-4Is were included and other medications or dietary factors were not controlled. Thus, some confounding factors are likely to have been overlooked. Third, no physical activity questionnaire was carried out during the observational period, and inconsistencies might exist between the estimations and the actual activities. Fourth, the limited sample size could have resulted in underestimation of the effects of the SNPs or other covariates. Fifth, 16 patients were excluded because of the lack of matched IPAQ data, and this might cause selection bias. Finally, we cannot exclude the possibility that another genetic locus that might be closely linked to the SNPs analyzed herein is responsible for the responsiveness to DPP-4Is.

In conclusion, the present results suggest that Japanese patients with type 2 diabetes might have a lower risk of being non-responders to DPP-4Is if they have a combination of the rs1800796 G allele and the rs2097677 A allele, and at the same time a certain level of physical activity. However, larger, controlled, prospective studies are required to confirm this.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 | Summary of the haplotype estimation of the two single nucleotide polymorphisms.