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

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INTRODUCTION
The prevalence of diabetes in the Western Pacific Region was reported to be 8.6% of adults, or 138 million, in 2013, and it has been estimated that it will rise to 11.1%, or 201 million, in 20351. Intensive lifestyle interventions to modify dietary and physical activity habits lead to weight reduction, delaying or preventing the onset of type 2 diabetes mellitus2. Weight changes through lifestyle modifications might depend on the

ABSTRACT
The beta-3 adrenergic receptor (ADRB3), primarily expressed in adipose tissue, is involved in the regulation of energy metabolism. The present study hypothesized that ADRB3 (Trp64Arg, rs4994) polymorphisms modulate the effects of lifestyle intervention on weight and metabolic parameters in patients with impaired glucose tolerance. Data were analyzed from 112 patients with impaired glucose tolerance in the Japan Diabetes Prevention Program, a lifestyle intervention trial, randomized to either an intensive lifestyle intervention group or usual care group. Changes in weight and metabolic parameters were measured after the 6-month intervention. The ADRB3 polymorphisms were determined using the polymerase chain reaction restriction fragment length polymorphism method. Non-carriers showed a greater weight reduction compared with the carriers in both the lifestyle intervention group and usual care group, and a greater increase of high-density lipoprotein cholesterol levels than the carriers only in the lifestyle intervention group. ADRB3 polymorphisms could influence the effects of lifestyle interventions on weight and lipid parameters in impaired glucose tolerance patients.
Effects are variable, and genetic factors might be involved. In the beta-3 adrenergic receptor (ADRB3), primarily expressed in adipose tissue, is involved in the regulation of energy metabolism. The polymorphism of ADRB3 in codon 64 (Trp64Arg; rs4994) is associated with abdominal obesity and insulin resistance syndrome. Obese diabetic and non-diabetic subjects with this polymorphism were found to be resistant to a low-calorie diet and exercise. Lifestyle interventions generally improve lipid profiles and reduce the risk of cardiovascular disease, but the effects are variable, and genetic factors might be involved. In the Finnish Diabetes Prevention Study, the −501A/C (rs26802) polymorphism in preproglucagon, but not ADRB3, modified the effect of total and moderate-to-vigorous physical activity on change in high-density lipoprotein cholesterol. It remained unclarified whether ADRB3 polymorphism modifies the effect of lifestyle intervention on weight reduction and metabolic parameters in subjects with impaired glucose tolerance. The aim of the present study was to evaluate the effects of gene–treatment interactions on weight and serum lipids in subjects with impaired glucose tolerance.

MATERIALS AND METHODS
Study design and participants
The study design, protocol, recruitment and interim results of the Japan Diabetes Prevention Program were described in detail previously. In short, the study was an unmasked, multicenter, randomized controlled trial carried out at 32 public health centers throughout Japan. Individuals with impaired glucose tolerance and aged 30–60 years were recruited through health checkups carried out at each health center. The definition of impaired glucose tolerance using the 75-g oral glucose tolerance test was based on the World Health Organization criteria. The exclusion criteria were as previously described. Participants were randomly assigned to either an intensive lifestyle intervention group (ILG) or a usual care group (UCG). Among a total of 304 patients recruited, just 110 patients (53 in the control group and 57 in the intervention group), who agreed to deoxyribonucleic acid analysis of ADRB3 polymorphism, were available for the present study.

Ethics
The study was approved by the ethical committee at the National Hospital Organization Kyoto Medical Center, Kyoto, Japan.

Intervention
The intervention program was described previously. Briefly, public health nurses or dieticians at each health center carried out lifestyle interventions individually. The goals of intervention were: (i) to reduce the initial bodyweight by 5% in overweight and obese participants, and (ii) to increase energy expenditure through promoting leisure time physical activity by 700 kcal per week. The participants in the UCG received only one group session providing advice on a healthy lifestyle for the prevention of type 2 diabetes mellitus at the outset. No individual guidance was given to the UCG during the study period.

Measures
Body mass index was calculated as the weight (kg)/height² (m²), measured with the participants wearing light clothes without shoes. Blood pressure was measured in the upper arm of seated participants. Waist circumference was measured at the umbilical level. After fasting for ≥12 h, blood was drawn from an antecubital vein. Plasma glucose, serum insulin and serum lipids, including high-density lipoprotein cholesterol (HDL-C) were measured at a nationally-certified central laboratory (SRL Inc., Tokyo, Japan). Genomic deoxyribonucleic acid was extracted from peripheral blood leukocytes. The Mva I polymorphisms (Trp64Arg; rs4994) of the ADRB3 gene were determined by polymerase chain reaction restriction fragment length polymorphism analysis, as previously described.

Statistical analysis
All data are presented as the mean ± standard deviation, and categorical variables are expressed as numbers counted. The three possible genotypic frequencies were evaluated by χ²-tests, and found to be in Hardy–Weinberg equilibrium. Differences in the baseline characteristics between participants in the control and intervention groups were evaluated by independent t-tests. Changes in each parameter from the baseline until after the 6-month intervention were evaluated using paired t-tests. The participants in each group were divided into two subgroups: carriers (Trp64/Arg64 and Arg64/Arg64) and non-carriers (Trp64/Trp64). Two main effects of interest (gene, intervention) and one interaction (gene × intervention) were tested. A P-value <0.05 was considered significant. Statistical analysis was carried out using the SPSS program version 20.0 (IBM SPSS, Tokyo, Japan).

RESULTS
The mean age and percentage of men were 51 ± 6 years and 40%, respectively. The distributions of Trp64/Trp64 homozygous, Trp64/Arg64 heterozygous, and Arg64/Arg64 homozygous were 80, 28 and 4, respectively. The frequencies were in Hardy–Weinberg equilibrium and consistent with a previous report on different Japanese populations. The allelic frequencies were similar across the ILG and UCG.

At the baseline (Table 1), there were no significant differences in bodyweight, body mass index, waist circumference, blood pressure, blood glucose or serum lipids between carriers and non-carriers. The non-carriers showed significantly greater reductions of weight and body mass index than the carriers. There was a significant interaction of lifestyle intervention on HDL-C level; that is, the non-carriers showed a significantly greater increase of HDL-C levels than carriers only in the ILG (P = 0.001). Except for weight and HDL-C, changes of other parameters were not significantly affected by Trp64Arg polymorphisms (Table 1).
### Table 1 | Baseline and 6-month follow-up data on anthropometric and metabolic values in the control and intervention groups by beta-3 adrenergic receptor polymorphism

| Parameters                         | Control group                  | Intervention group             | Gene-treatment interaction |
|-----------------------------------|--------------------------------|--------------------------------|----------------------------|
|                                   | Baseline‡ | 6 months | Change | n | P† | Baseline | 6 months | Change | n | P | Carrier | Intervention | Interaction |
| Weight (kg)                       | Non-carrier 644 ± 11.1 | 633 ± 10.5 | −11 ± 20 | 37 | 0.001 | 612 ± 12.8 | 590 ± 12.6 | −22 ± 23 | 41 | <0.001 | 0.043 | 0.100 | 0.496 |
|                                   | Carrier 643 ± 13.2 | 639 ± 12.7 | −04 ± 21 | 16 | 0.301 | 621 ± 11.6 | 611 ± 12.0 | −10 ± 22 | 16 | 0.102 | 0.059 | 0.55 | 0.058 |
| Body mass index (kg/m²)           | Non-carrier 25.1 ± 33 | 24.7 ± 32 | −0.4 ± 11 | 37 | 0.002 | 24.2 ± 3.8 | 23.4 ± 2.8 | −0.9 ± 0.9 | 41 | <0.001 | 0.039 | 0.055 | 0.55 |
|                                   | Carrier 24.5 ± 43 | 24.3 ± 43 | −0.2 ± 08 | 16 | 0.368 | 24.1 ± 2.8 | 23.7 ± 2.8 | −0.4 ± 0.8 | 16 | 0.081 | 0.542 | 0.810 | 0.545 |
| Waist circumference (cm)          | Non-carrier 86.3 ± 90 | 84.6 ± 87 | −1.7 ± 43 | 37 | 0.025 | 83.3 ± 10.7 | 80.8 ± 10.7 | −2.4 ± 47 | 41 | 0.002 | 0.542 | 0.810 | 0.545 |
|                                   | Carrier 84.8 ± 95 | 83.2 ± 80 | −1.9 ± 45 | 16 | 0.297 | 83.9 ± 8.9 | 81.6 ± 8.8 | −2.3 ± 39 | 16 | 0.028 | 0.542 | 0.828 | 0.874 |
| Systolic blood pressure (mmHg)    | Non-carrier 127 ± 15 | 128 ± 17 | 1 ± 16 | 36 | 0.833 | 132 ± 15 | 128 ± 17 | −4 ± 15 | 39 | 0.081 | 0.520 | 0.539 | 0.719 |
|                                   | Carrier 134 ± 22 | 135 ± 19 | 1 ± 14 | 16 | 0.745 | 138 ± 16 | 134 ± 20 | −3 ± 17 | 16 | 0.487 | 0.542 | 0.828 | 0.874 |
| Diastolic blood pressure (mmHg)   | Non-carrier 78 ± 9 | 77 ± 9 | −0 ± 9 | 36 | 0.774 | 81 ± 10 | 78 ± 10 | −3 ± 10 | 39 | 0.051 | 0.542 | 0.828 | 0.874 |
|                                   | Carrier 83 ± 11 | 82 ± 13 | −1 ± 9 | 16 | 0.700 | 84 ± 11 | 83 ± 11 | −2 ± 6 | 16 | 0.253 | 0.902 | 0.173 | 0.257 |
| Fasting plasma glucose (mmol/L)   | Non-carrier 109 ± 10 | 106 ± 10 | −3 ± 8 | 37 | 0.020 | 106 ± 9 | 103 ± 11 | −4 ± 9 | 42 | 0.011 | 0.354 | 0.176 | 0.492 |
|                                   | Carrier 109 ± 11 | 108 ± 10 | −1 ± 11 | 16 | 0.666 | 109 ± 8 | 103 ± 10 | −6 ± 11 | 16 | 0.045 | 0.867 | 0.458 | 0.038 |
| 2-h plasma glucose (mmol/L)       | Non-carrier 160 ± 15 | 142 ± 41 | −18 ± 41 | 37 | 0.009 | 165 ± 16 | 132 ± 28 | −33 ± 27 | 42 | <0.001 | 0.016 | 0.867 | 0.458 | 0.038 |
|                                   | Carrier 167 ± 18 | 150 ± 23 | −17 ± 29 | 16 | 0.034 | 160 ± 12 | 139 ± 35 | −22 ± 33 | 16 | 0.020 | 0.058 | 0.725 | 0.134 |
| Total cholesterol (mmol/L)        | Non-carrier 210 ± 34 | 207 ± 34 | −3 ± 26 | 37 | 0.451 | 221 ± 34 | 214 ± 30 | −7 ± 25 | 42 | 0.087 | 0.091 | 0.364 | 0.764 |
|                                   | Carrier 214 ± 29 | 222 ± 34 | 8 ± 26 | 16 | 0.249 | 219 ± 35 | 207 ± 34 | 1 ± 33 | 16 | 0.887 | 0.867 | 0.458 | 0.038 |
| HDL cholesterol (mmol/L)          | Non-carrier 59 ± 18 | 57 ± 19 | −2 ± 11 | 37 | 0.220 | 58 ± 14 | 61 ± 18 | 3 ± 8 | 42 | 0.016 | 0.867 | 0.458 | 0.038 |
|                                   | Carrier 59 ± 16 | 61 ± 18 | 2 ± 7 | 16 | 0.272 | 56 ± 16 | 55 ± 13 | 0 ± 8 | 16 | 0.837 | 0.058 | 0.725 | 0.134 |
| Triglycerides (mmol/L)            | Non-carrier 134 ± 96 | 137 ± 97 | 4 ± 93 | 37 | 0.774 | 125 ± 71 | 106 ± 72 | −19 ± 60 | 42 | 0.041 | 0.058 | 0.725 | 0.134 |
|                                   | Carrier 128 ± 59 | 135 ± 74 | 7 ± 61 | 16 | 0.647 | 100 ± 43 | 120 ± 70 | 21 ± 51 | 16 | 0.124 |

Data are means ± standard deviation. ‡Baseline vs 6 months. †There were no significant differences in any of the baseline variables between the control and intervention groups. HDL, high-density lipoprotein.
DISCUSSION
The present study suggests that Trp64Arg polymorphism might mediate the HDL-C levels in a minimal intervention with modest weight loss in patients with impaired glucose tolerance. Difficulties in weight reduction seen in the carriers in the present study are consistent with previous reports on obese subjects with or without type 2 diabetes mellitus. Defects in ADRB binding, signal transduction or regulatory mechanisms could result in a lower resting metabolic rate and diminished lipolysis in visceral adipose tissue, explaining the poor response to lifestyle intervention. However, there were some studies that failed to observe difficulties in weight reduction in carriers. Although the reason for the discrepancies among the studies is not clear, differences in the study participants (obesity alone, obesity with impaired glucose tolerance and type 2 diabetes mellitus) and study design might be associated.

The present study also showed a gene–treatment interaction on HDL-C levels. The Arg64 allele was cross-sectionally associated with a decreased HDL-C level in obese Japanese people and a Kyrgyz population, although a few studies described conflicting data—no association with HDL-C or association with higher HDL-C levels was found in Caucasian-Brazilian patients with type 2 diabetes. An increase of HDL-C levels is important for preventing the development of diabetes mellitus and atherosclerosis. Thus, the present findings could be valuable in providing an effective strategy to increase HDL-C in impaired glucose tolerance patients.

The strengths of the present study include its real-world setting and it being a randomized controlled trial. However, the study had several limitations, including the small sample size and the multiple hypothesis testing. These limitations should be addressed in future research.

In conclusion, these findings suggest that Trp64Arg polymorphism modulates weight change and the HDL-C response to lifestyle intervention in patients with impaired glucose tolerance. This genetic information could help identify individuals who require intensive lifestyle intervention.

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DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Chan JC, Cho NH, Tajima N, et al. Diabetes in the Western pacific region-past, present and future. Diabetes Res Clin Pract 2014; 103: 244–255.
2. Johnson M, Jones R, Freeman C, et al. Can diabetes prevention programmes be translated effectively into real-world settings and still deliver improved outcomes? A synthesis of evidence. Diabet Med 2013; 30: 3–15.
3. Deram S, Villares SM. Genetic variants influencing effectiveness of weight loss strategies. Arq Bras Endocrinol Metabol 2009; 53: 129–138.
4. Mund RA, Frishman WH. Brown adipose tissue thermogenesis: β3-adrenoceptors as a potential target for the treatment of obesity in humans. Cardiol Rev 2013; 21: 265–269.
5. Walston J, Silver K, Bogardus C, et al. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3-adrenergic-receptor gene. N Engl J Med 1995; 333: 343–347.
6. Sakane N, Yoshida T, Umekawa T, et al. Beta 3-adrenergic-receptor polymorphism: a genetic marker for visceral fat obesity and the insulin resistance syndrome. Diabetologia 1997; 40: 200–204.
7. 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.
8. Sakane N, Yoshida T, Umekawa T, et al. Effects of Trp64Arg mutation in the beta 3-adrenergic receptor gene on weight loss, body fat distribution, glycemic control, and insulin resistance in obese type 2 diabetic patients. Diabetes Care 1997; 20: 1887–1890.
9. Pollin TI, Isaakova T, Jablonski KA, et al. Genetic modulation of lipid profiles following lifestyle modification or metformin treatment: the Diabetes Prevention Program. PLoS Genet 2012; 8: e1002895.
10. Kilpeläinen TO, Lakka TA, Laaksonen DE, et al. Interaction of single nucleotide polymorphisms in ADRB2, ADRB3, TNF, IL6, IGFR1, LIPC, LEPR, and GHRL with physical activity on the risk of type 2 diabetes mellitus and changes in characteristics of the metabolic syndrome: The Finnish Diabetes Prevention Study. Metabolism 2008; 57: 428–436.
11. Gomyo M, Sakane N, Kamae I, et al. Effects of sex, age, and BMI on screening tests for impaired glucose tolerance. Diabetes Res Clin Pract 2004; 64: 129–136.
12. Sakane N, Sato J, Tsushita K, et al. Prevention of type 2 diabetes in a primary healthcare setting: three-year results of lifestyle intervention in Japanese subjects with impaired glucose tolerance. BMC Public Health 2011; 11: 40.
13. Kuzuya T, Nakagawa S, Satoh J, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. Diabetes Res Clin Pract 2002; 55: 65–85.
14. Takeuchi S, Katoh T, Yamauchi T, et al. ADRB3 polymorphism associated with BMI gain in Japanese men. Exp Diabetes Res 2012; 2012: 973561.
15. Kurokawa N, Nakai K, Kameo S, et al. Association of BMI with the beta3-adrenergic receptor gene polymorphism in Japanese: meta-analysis. Obes Res 2001; 9: 741–745.
16. Shiwaku K, Nogi A, Anuurad E, et al. Difficulty in losing weight by behavioral intervention for women with Trp64Arg polymorphism of the β3-adrenergic receptor gene. Int J Obes Relat Metab Disord 2003; 27: 1028–1036.

17. Piétri-RouxF F, St John Manning B, Gros J, et al. The biochemical effect of the naturally occurring Trp64→Arg mutation on human β3-adrenoceptor activity. Eur J Biochem 1997; 247: 1174–1179.

18. Umekawa T, Yoshida T, Sakane N, et al. Trp64Arg mutation of β3-adrenoceptor gene deteriorates lipolysis induced by β3-adrenoceptor agonist in human omental adipocytes. Diabetes 1999; 48: 117–120.

19. Kuriyama S, Shimazu T, Hozawa A, et al. No effect of the Trp64Arg variant of the β3-adrenergic receptor gene on weight loss by diet and exercise intervention among Japanese adults. Metabolism 2008; 57: 1570–1575.

20. Tahara A, Osaki Y, Kishimoto T. Effect of the β3-adrenergic receptor gene polymorphism Trp64Arg on BMI reduction associated with an exercise-based intervention program in Japanese middle-aged males. Environ Health Prev Med 2010; 15: 392–397.

21. Brondani LA, Duarte GC, Canani LH, et al. The presence of at least three alleles of the ADRB3 Trp64Arg (C/T) and UCP1-3826A/G polymorphisms is associated with protection to overweight/obesity and with higher high-density lipoprotein cholesterol levels in Caucasian-Brazilian patients with type 2 diabetes. Metab Syndr Relat Disord 2014; 12: 16–24.

22. Kim JY, Lee SS. The effects of uncoupling protein 1 and beta3-adrenergic receptor gene polymorphisms on weight loss and lipid profiles in obese women. Int J Vitam Nutr Res 2010; 80: 87–96.

23. Mirrakhimov AE, Kerimkulova AS, Lunegova OS, et al. An association between TRP64ARG polymorphism of the B3 adrenoreceptor gene and some metabolic disturbances. Cardiovasc Diabetol 2011; 10: 89.

24. Nishimura R, Sone H, Nakagami T, et al. Importance of high-density lipoprotein cholesterol control during pravastatin treatment in hypercholesterolemic Japanese with type 2 diabetes mellitus: a post hoc analysis of MEGA study. Diabetes Res Clin Pract 2013; 100: e31–e33.