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Interaction of single nucleotide polymorphisms in *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *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

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Abstract

Single nucleotide polymorphisms (SNPs) in the *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, and *GHRL* genes were associated with the conversion from impaired glucose tolerance (IGT) to type 2 diabetes mellitus (T2D) in the Finnish Diabetes Prevention Study (DPS). In this study, we determined whether polymorphisms in these genes modified the effect of changes in physical activity (PA) on the risk of T2D in the DPS. Moreover, we assessed whether the polymorphisms modified the effect of changes in PA on changes in measures of body fat, serum lipids, and blood pressure during the first year of the follow-up of the DPS. Overweight subjects with IGT (n = 487) were followed for an average of 4.1 years, and PA was assessed annually with a questionnaire. The interactions of the polymorphisms with changes in total and moderate-to-vigorous PA on the conversion to T2D during the 4.1-year follow-up were assessed using Cox regression with adjustments for the other components of the intervention (dietary changes, weight reduction). Univariate analysis of variance was used to assess interactions on changes in continuous variables during the first year of the follow-up. No interaction between the polymorphisms and PA on the conversion to T2D was found. The Leu72Met (rs696217) polymorphism in *GHRL* modified the effect of moderate-to-vigorous PA on changes in weight and waist circumference, the −501A/C (rs26802) polymorphism in *GHRL* modified the effect of total and moderate-to-vigorous PA on change in high-density lipoprotein cholesterol, and the Lys109Arg (rs1137100) polymorphism in *LEPR* modified the effect of total PA on change in blood pressure. In conclusion, genetic variation may modify the magnitude of the beneficial effects of PA on characteristics of the metabolic syndrome in persons with IGT.

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1. Introduction

Genes and lifestyle interact in the development of type 2 diabetes mellitus (T2D). In the Finnish Diabetes Prevention Study (DPS), the 3-year lifestyle intervention led to a 58% reduction in the risk of T2D among 522 overweight subjects with an impaired glucose tolerance (IGT) [1]. In the genetic association analyses, polymorphisms in 12 genes, including peroxisome proliferator-activated receptor γ (*PPARG*) [2], α2b-adrenergic receptor (*ADRA2B*) [3], β2-adrenergic receptor (*ADRB2*) and β3-adrenergic receptor (*ADRB3*) [4], tumor necrosis factor α (*TNF*) and interleukin-6 (*IL6*) [5], insulin-like growth factor 1 receptor (*IGF1R*) [6], hepatic lipase (*LIPC*) [7], leptin receptor (*LEPR*) [8], sulphonylurea receptor 1 (*ABCC8*) [9], glucose transporter 2 (*SLC2A2*) [10], and ghrelin (*GHRL*) [11], were associated with the risk of developing T2D in the DPS. However, with many of these genes, the association with T2D was more pronounced in either the intervention or the control group of the DPS, indicating a gene-lifestyle interaction [2-5,7-11]. Whether there were interactions between the genetic variants and more specific changes in lifestyle, such as physical activity (PA) or diet, on the risk of T2D was not assessed.

Gene variants may contribute to the magnitude of the effect of PA on the risk of T2D [12]. In the DPS, the change in the weekly amount of PA was a strong predictor of the conversion from IGT to T2D; the subjects who increased their PA most (ie, were in the upper third of the change) were 66% less likely to develop T2D than those in the lower third, independent of changes in diet and body weight [13]. Interestingly, we have recently shown that polymorphisms in the *ADRA2B*, *ABCC8*, and *SLC2A2* genes modified the effect of PA on the risk of developing T2D [14,15]. Our unpublished findings also indicate that polymorphisms in the *PPARG* gene may modify the effect of PA on the risk of developing T2D. Whether the 8 other genes that have been associated with T2D in the DPS, including *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, and *GHRL*, also interacted with PA on the conversion to T2D has not yet been analyzed.

Apart from being obese and having IGT, more than 70% of the participants of the DPS have the metabolic syndrome, characterized by abdominal obesity, dyslipidemia, and elevated blood pressure [16]. Physical activity exerts favorable effects on the characteristics of the metabolic syndrome [17], but the magnitude of the effects is modified by genetic variation [12]. Therefore, the study of interactions between changes in PA and the characteristics of the metabolic syndrome in the DPS is warranted.

We examined whether polymorphisms in the *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, and *GHRL* genes interacted with changes in total or moderate-to-vigorous PA on the conversion to T2D during an average follow-up of 4.1 years in the DPS. We also assessed whether the polymorphisms of these genes modified the effect of PA on the changes in the features of the metabolic syndrome, that is, weight, waist circumference, serum lipids, and blood pressure, during the first year of the intensive intervention period of the DPS.

2. Subjects and methods

2.1. Study design and population

The present study is a post hoc analysis of the Finnish DPS, a multicenter randomized controlled trial on the effects of lifestyle modification, including an increase in PA, favorable dietary changes, and weight reduction, on the prevention of T2D in high-risk individuals. The design of the DPS has been described in detail elsewhere [18,19].

In brief, altogether, 522 middle-aged (mean age, 55 years) and overweight (mean body mass index [BMI], 31 kg/m^2) Finnish individuals with IGT were randomly assigned either to an intervention group (n = 265) or a control group (n = 257). *Impaired glucose tolerance* was defined as a plasma glucose concentration of 7.8 to 11.0 mmol/L 2 hours after the oral administration of 75 g of glucose in those whose plasma glucose concentration after an overnight fast was less than 7.8 mmol/L [20].

The intervention group received individualized counseling aimed at increasing PA, reducing body weight, reducing the intake of total and saturated fat, and increasing the intake of dietary fiber. Endurance exercise, including walking, jogging, swimming, aerobic ball games, and skiing, was encouraged. Supervised, progressive, individually tailored, circuit-type resistance training beginning 4 to 6 months after the randomization was offered to the intervention group free of charge in 3 of 5 centers. Lifestyle PA was also promoted. The control group received general oral and written information about diet and exercise at baseline, but no specific individualized programs were offered to them [19].

2.2. Genotyping

The polymorphisms that were included in the present study are listed in Table 1. The genotyping for the polymorphisms of the *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, and *LIPC* genes was performed in 490 subjects [4-7]. The genotyping for the polymorphisms of the *LEPR* and *GHRL* genes was performed in 507 subjects [8,11,21]. All polymorphisms were screened by the restriction fragment length polymorphism method [4-8,11,21], except for the −174C/G (rs1800795) polymorphism of *IL6* that was determined by the single-strand conformation polymorphism technique, as previously described [5].

2.3. Assessment of PA and diet

All subjects completed a PA questionnaire at baseline and at each annual visit. Physical activity was assessed using the validated Kuopio Ischemic Heart Disease Risk Factor Study 12-month Leisure Time Physical Activity Questionnaire [22]. The questionnaire provides detailed quantitative information on the duration, frequency, and mean intensity

Table 1
The genes and SNPs investigated in this study

Gene	SNP
ADRB2	Gln27Glu (rs1042714)
ADRB3	Trp64Arg (rs4994)
TNF	−308G/A (rs1800629)
IL6	−174C/G (rs1800795)
IGF1R	Glu1013Glu (rs2229765)
LIPC	−250G/A (rs2070895)
LEPR	Lys109Arg (rs1137100)
	Gln223Arg (rs1137101)
	3′ UTR deletion/insertion (no rs number)
GHRL	−604G/A (rs27647)
	−501A/C (rs26802)
	−473G/A (no rs number)
	Arg51Gln (no rs number)
	Leu72Met (rs696217)
	Gln90Leu (rs4684677)

UTR indicates untranslated region.

of the most common lifestyle and structured PA as recalled over the previous 12 months [23]. *Moderate-to-vigorous PA* was defined as ≥3.5 METs and *low-intensity PA* as <3.5 METs (*1 MET* is defined as metabolic expenditure at rest, corresponding to an oxygen uptake of 3.5 mL O_2/kg). Common moderate-to-vigorous PAs included moderate- to high-intensity walking, bicycling, swimming, resistance training, skiing, jogging, ball games, and lifestyle activities such as chopping wood or clearing brush. Common low-intensity activities included low-intensity walking or bicycling, yard work and gardening, and picking berries and mushrooms [13]. The assessment of diet by a 3-day food diary has been described previously [19]. The average intakes of energy, total fat, saturated fat, and dietary fiber were calculated at baseline and at 1-, 2-, and 3-year visits of the intervention period using a dietary analysis program developed at the National Public Health Institute, Helsinki, Finland [24].

2.4. Measurement of glucose homeostasis

All subjects underwent an oral glucose tolerance test (OGTT) at baseline and at each annual visit, as described previously [1,18]. Serum insulin concentration was measured by a radioimmunoassay (Pharmacia, Uppsala, Sweden).

2.5. Anthropometric measurements

Body weight and height were measured annually, and BMI was calculated as weight divided by height squared (kilograms per square meter). Waist circumference was measured midway between the lowest rib and iliac crest.

2.6. Measurement of serum lipids and blood pressure

Serum levels of high-density lipoprotein (HDL) cholesterol and triglycerides were measured by an enzymatic assay at the National Public Health Institute, Analytical Biochemistry Laboratory, Helsinki. Blood pressure was measured twice from the right arm using a standard sphygmomanometer after 10 minutes of rest with the subject in a sitting position. The mean of the 2 measurements was used in the calculations.

2.7. Definition of T2D

Diabetes was defined according to the 1985 criteria of the World Health Organization [20] as either a fasting plasma glucose concentration ≥7.8 mmol/L or a plasma glucose concentration ≥11.1 mmol/L 2 hours after a 75-g oral glucose challenge. Participants were asked to fast and to refrain from strenuous exercise for 12 hours before the OGTT. If the diagnosis of diabetes was not confirmed by a second OGTT, the subject continued in the study [1].

Altogether, 86 cases of incident diabetes were diagnosed when the original trial ended after an average follow-up of 3.2 years [1]. To increase statistical power for detecting interactions between SNPs and PA on the conversion to T2D, we extended the follow-up by 1 year (average, 4.1 years) [13,24], during which the participants still followed their randomized intervention program. Thus, the final number of participants who developed T2D was 116. Of all the 522 participants of the DPS, 487 gave sufficient information on changes in PA and are included in the present analyses. Of these 487 subjects, 107 developed T2D during the 4.1-year follow-up.

2.8. Statistical analyses

Variables were statistically transformed to approximate normality when required. Differences in the levels of total, moderate-to-vigorous, and low-intensity PA as well as in clinical characteristics among genotypes at baseline were evaluated by the univariate analysis of variance with adjustments for age, sex, and BMI (not for anthropometric variables).

The changes in total, moderate-to-vigorous, and low-intensity PA during the trial were calculated by subtracting the baseline PA values (in hours per week) from averaged annual PA values during the 4.1-year follow-up [13]. The changes in total, moderate-to-vigorous, and low-intensity PA during the first year of the follow-up were calculated by subtracting the first-year PA values from the baseline values. Changes in dietary, biochemical, and anthropometric measures during the trial were calculated similarly with the PA values. However, the data for nutrient intakes were available from the first 3 years of the follow-up only [24]. The intakes of total and saturated fat and fiber were adjusted by daily energy intake with linear regression analysis before further statistical analyses [25].

The association of the polymorphisms in the *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, and *GHRL* genes with the conversion to T2D according to changes in PA in the combined intervention and control groups of the DPS was assessed using Cox proportional hazards models with relevant adjustments. The interaction of the polymorphisms with weight changes on the conversion to T2D was similarly tested. The association of the polymorphisms with changes

in anthropometric characteristics, serum lipids, and blood pressure according to changes in PA was evaluated by the univariate analysis of variance, general linear model. *Statistical significance* was defined as $P < .05$. However, as we performed the interaction analyses for 5 different clinical variables (weight, waist circumference, HDL cholesterol, serum triglycerides, and systolic blood pressure), a Bonferroni correction factor of 5 was applied to the nominal P values in the analyses of the interactions between PA and SNPs on changes in the characteristics of the metabolic syndrome. Analyses were performed with SPSS 11.5 for Windows (Chicago, IL).

3. Results

3.1. Baseline

The genotype frequencies of all SNPs were in Hardy-Weinberg equilibrium ($P > .05$). The baseline characteristics of the 487 study participants who provided information on changes in PA are described in Table 2. The level of total PA was higher in the carriers of the Met72 allele of the Leu72Met (rs696217) polymorphism in *GHRL* than in those who were Leu72 homozygous (7.9 ± 6.4 vs 7.0 ± 5.9 h/wk, $P = .032$). Similarly, the baseline level of total PA was higher in the carriers of the A allele of the −501A/C (rs26802) polymorphism in *GHRL* than in those carrying the common homozygous genotype (7.6 ± 6.4 vs 6.9 ± 5.8 h/wk, $P = .034$). The baseline level of moderate-to-vigorous PA was lower in the carriers of the A allele of the Glu1013Glu (rs2229765) polymorphism in *IGF1R* than in the carriers of the common homozygous genotype among men (4.3 ± 3.0 vs 3.0 ± 3.4 h/wk, $P = .003$) but not in women ($P = .679$) ($P = .008$ for interaction between sex and genotype). No other differences in the level of total, moderate-to-vigorous, or low-intensity PA according to the polymorphisms in *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR* or *GHRL* at baseline were found (data not shown). The baseline differences in clinical characteristics among the genotypes have been reported previously [4-8,11,23]. Weight or waist circumference did not vary according to the Leu72Met polymorphism in *GHRL* [11]; neither did serum HDL cholesterol according to the −501A/C polymorphism in *GHRL* (data not shown) nor systolic blood pressure according to the Lys109Arg (rs1137100) polymorphism in *LEPR* (data not shown).

Table 2
Baseline characteristics of participants in the Finnish DPS

Characteristic	Level at baseline
n	487
Sex (male/female)	162/325
Age (y)	55.4 ± 7.0
Weight (kg)	86.3 ± 14.3
BMI (kg/m^2)	31.3 ± 4.5
Waist circumference (cm)	101.2 ± 11.0
Fasting plasma glucose (mmol/L)	6.1 ± 0.7
2-h plasma glucose (mmol/L)	8.9 ± 1.5
Fasting serum insulin (mU/L)	14.7 ± 7.3
2-h serum insulin (mU/L)	95.4 ± 65.5
Energy intake (kcal)	1767 ± 527
Total fat intake, energy adjusted (g)	72.2 ± 12.7
Saturated fat intake, energy adjusted (g)	32.8 ± 8.2
Fiber intake, energy adjusted (g)	19.9 ± 6.6
Total PA (h/wk)[a]	5.7 (3.1-9.3)
Moderate-to-vigorous PA (h/wk)	1.7 (0.5-4.0)
Low-intensity PA (h/wk)	3.0 (1.2-5.9)

Data are means ± SD or medians (interquartile ranges) and are given for participants who provided sufficient information on PA.

[a] Total PA is the sum of moderate-to-vigorous and low-intensity PA.

3.2. Risk of T2D

No statistically significant interactions between the polymorphisms in *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, or *GHRL* and changes in total or moderate-to-vigorous leisure-time PA on the risk of T2D during the 4.1-year follow-up were found in the combined intervention and control groups of the DPS (data not shown). Neither did we find significant interactions between these polymorphisms and changes in body weight on the risk of T2D during the 4.1-year follow-up (data not shown).

3.3. Anthropometric characteristics, serum lipids, and blood pressure

The Leu72Met (rs696217) polymorphism in the *GHRL* gene modified the effect of moderate-to-vigorous PA on the changes in body weight and waist circumference during the first study year of the DPS (Fig. 1) ($P = .001$ and $P = .006$, respectively, for the interaction between PA change and Leu72Met genotype; Bonferroni-corrected $P = .005$ and $P = .030$). Among the carriers of the Met72 allele, body weight and waist circumference decreased more in those who were in the middle or upper third of the change in moderate-to-vigorous PA, whereas the Leu72 homozygotes did not respond to changes in PA (Fig. 1).

The −501A/C (rs26802) polymorphism in the *GHRL* gene modified the effect of total and moderate-to-vigorous PA on the change in serum HDL cholesterol concentration during the first study year of the DPS ($P = .005$ and $P = .024$, respectively, for interaction; Bonferroni-corrected $P = .025$ and $P = .120$) (Fig. 2). The HDL cholesterol concentration increased in the participants homozygous for AA who increased total and moderate-to-vigorous PA, but no increase was seen among the carriers of the C allele (Fig. 2).

The Lys109Arg (rs1137100) polymorphism in the *LEPR* gene modified the effect of the change in total PA on the change in systolic blood pressure during the first study year ($P = .017$, Bonferroni-corrected $P = .085$) (Fig. 3). Systolic blood pressure decreased only in the Lys109 homozygotes who increased their PA, whereas the carriers of the Arg109 allele did not respond to changes in PA (Fig. 3).

No differences were found in changes in total or moderate-to-vigorous PA during the first study year of the

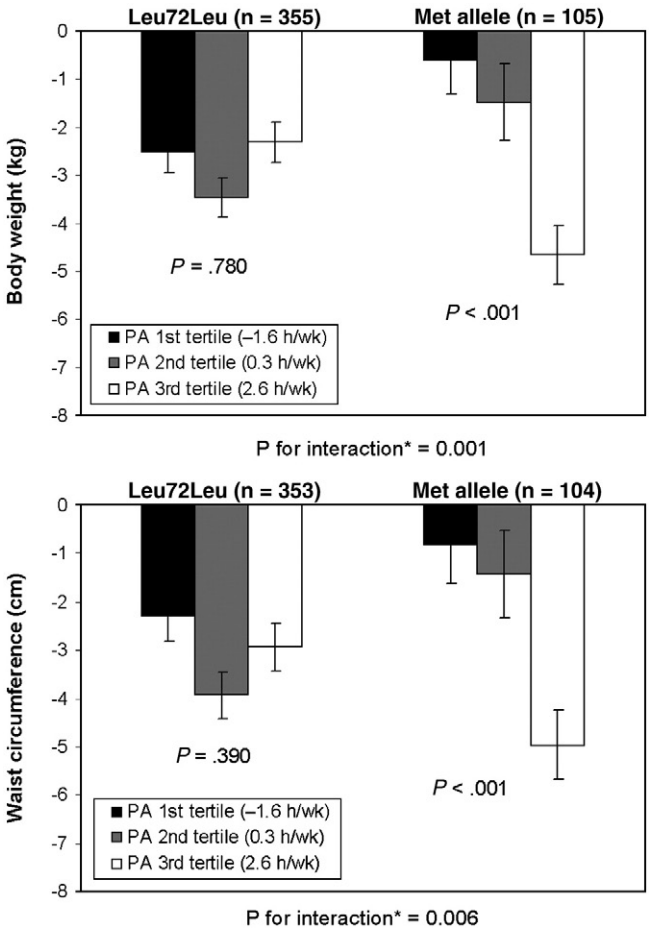

Fig. 1. Association of the Leu72Met (rs696217) polymorphism in *GHRL* with the changes in body weight and waist circumference according to tertiles of the change in moderate-to-vigorous PA during the first year of the follow-up of the Finnish DPS. The median of each third of PA change is shown. *P* values are for the linear trend. The values are adjusted for the baseline value of the dependent variable, age, sex, group, baseline value of moderate-to-vigorous PA, and baseline values and 1-year changes in low-intensity PA and energy intake. *Adjusted interaction between moderate-to-vigorous PA (3 groups) and the Leu72Met polymorphism (2 groups) on the change in the dependent variable.

DPS according to the Leu72Met or the −501A/C polymorphism in *GHRL* or the Lys109Arg polymorphism in *LEPR* (data not shown).

4. Discussion

Polymorphisms in the *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, and *GHRL* genes did not modify the association of changes in PA with the risk of developing T2D in the DPS. However, the Leu72Met (rs696217) polymorphism in *GHRL* modified the effect of moderate-to-vigorous PA on the changes in weight and waist circumference, the −501A/C (rs26802) polymorphism in *GHRL* modified the effect of total and moderate-to-vigorous PA on the change in HDL cholesterol concentration, and the Lys109Arg (rs1137100) polymorphism in *LEPR* modified the effect of total PA on the change in systolic blood pressure.

Although the association of polymorphisms in the *ADRB3*, *TNF*, *IL6*, *LIPC*, and *GHRL* genes with the risk of T2D was more pronounced in the intervention group [4,5,7,11] and the association of polymorphisms in the *LEPR* gene was only found in the control group of the DPS [8], we were not able to detect interactions between these polymorphisms and changes in PA or body weight on the risk of T2D during the 4.1-year follow-up. Therefore, the polymorphisms may have interacted with other lifestyle changes, such as diet, or with the lifestyle intervention as a whole. No other reports are available on interactions between variants in these genes and PA on the risk of developing T2D. However, polymorphisms in *IL6*, *LIPC*, and *LEPR* have been reported to modify the effects of aerobic exercise

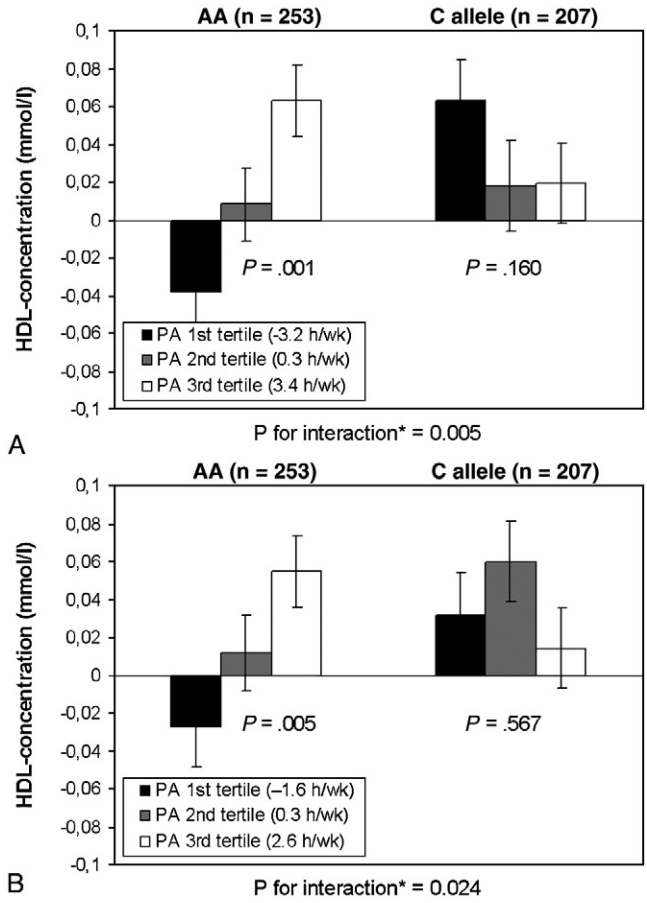

Fig. 2. Association of the −501A/C (rs26802) polymorphism in *GHRL* with the change in concentrations of HDL cholesterol according to tertiles of the change in (A) total PA and (B) moderate-to-vigorous PA during the first year of the follow-up of the Finnish DPS. The median of each tertile of PA change is shown. *P* values are for the linear trend. The values are adjusted for the baseline HDL cholesterol, age, sex, group, baseline values and 1-year changes in BMI and fat intake, and baseline value of (A) total PA or (B) moderate-to-vigorous PA. Panel B is also adjusted for baseline value and 1-year changes in low-intensity PA. *Adjusted interaction between PA (3 groups) and the −501 A/C polymorphism (2 groups) on the change in HDL concentration.

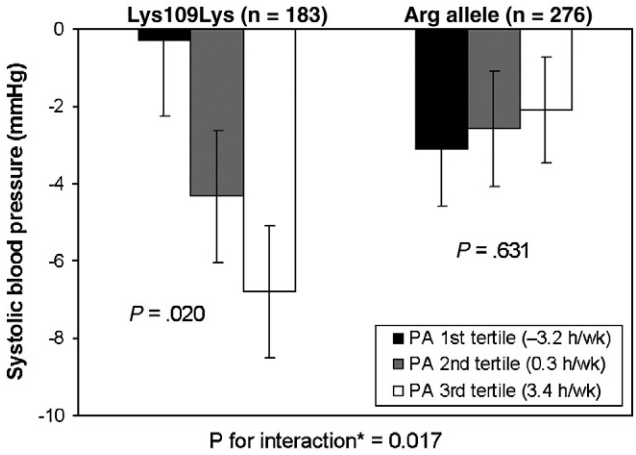

Fig. 3. Association of the Lys109Arg (rs1137100) polymorphism in *LEPR* with the change in systolic blood pressure according to tertiles of the change in total PA during the first year of the follow-up of the Finnish DPS. The median of each third of PA change is shown. *P* values are for the linear trend. The values are adjusted for the baseline systolic blood pressure, age, sex, group, baseline value of total PA, and baseline values and changes in body mass index. *Adjusted interaction between total PA (3 groups) and the Lys109Arg polymorphism (2 groups) on the change in systolic blood pressure.

training on glucose homeostasis [26-28]. Our study may have been underpowered to detect interactions between polymorphisms in *IL6*, *LIPC*, and *LEPR* and changes in PA on the risk of T2D. Further studies are required to elucidate whether PA or some other specific lifestyle changes modify the risk of T2D associated with genotypes of *ADRB3*, *TNF*, *IL6*, *LIPC*, *LEPR*, and *GHRL*.

More than 70% of the participants of the DPS fulfilled the WHO diagnostic criteria for the metabolic syndrome, a clustering of abdominal obesity, dyslipidemia, and hypertension [16]. Physical activity improves many of the characteristics of this syndrome [17]. The magnitude of the beneficial changes evoked by PA varies between individuals, and some of these differences are due to interactions between genetic variants and PA [12]. Three such interactions were detected in the present study. Firstly, the Leu72Met polymorphism in *GHRL* modified the effect of moderate-to-vigorous PA on the changes in measures of overweight. Increased PA resulted in a decrease in body weight and waist circumference in the carriers of the Met72 allele but not in the Leu72 homozygotes. The interactions remained statistically significant after correction for multiple testing. Secondly, the −501A/C polymorphism in *GHRL* modified the effect of PA on the change in serum HDL cholesterol concentration. In the AA homozygotes of the −501A/C polymorphism who increased PA, HDL cholesterol levels increased, whereas no such changes were found among the carriers of the C allele. Thirdly, the Lys109Arg polymorphism in *LEPR* modified the effect of total PA on the change in systolic blood pressure. Increase in PA led to a decrease in blood pressure only in the Lys109 homozygotes, whereas the carriers of the Arg109 allele did not respond to PA.

No earlier reports exist on interaction between variants in the *GHRL* gene and PA on changes in measures of overweight. Ghrelin binds to the growth hormone (GH) secretagogue receptor, releasing GH to systemic circulation [29]. As GH has lipolytic activity, increased GH release through ghrelin action could lead to decreased adiposity [30]. Furthermore, ghrelin affects energy balance by stimulating appetite and increasing food intake [31]. The levels of ghrelin increase in relation to decreases in body weight, acting possibly as a compensatory signal to restore body weight [31].

Similarly with ghrelin, PA of moderate-to-vigorous intensity stimulates GH release [32]. However, short-term exercise does not seem to affect ghrelin concentrations, indicating that ghrelin is not involved in the exercise-induced stimulation of GH secretion [33]. Long-term aerobic exercise training increases ghrelin levels, but only when weight loss is produced [34,35]. However, even if the effect of PA on total ghrelin levels seems small, a 5-day aerobic exercise program was recently reported to increase the proportion of biologically active acylated ghrelin in blood [36]. Ghrelin circulates both in acylated and deacyl forms; but only acylated ghrelin binds to GH secretagogue receptor, affecting GH release and energy balance [37]. Moreover, 2 studies have reported that changes in ghrelin levels were related to changes in fat-free mass but not in fat mass during weight loss [38,39]. Physical activity could thus indirectly affect total ghrelin levels by maintaining or increasing fat-free mass. Unfortunately, ghrelin levels were not measured in our study.

Interestingly, the baseline level of total PA differed among the genotypes of the Leu72Met polymorphism, suggesting that the carriers of the Met72 allele adopt a higher level of activity than those with the Leu72 homozygous genotype. Indeed, some evidence indicates that centrally administered ghrelin decreases spontaneous PA in rats [40]. In the DPS, the interactions between the Leu72Met polymorphism and PA on measures of anthropometry were, however, not affected by adjustment for the baseline level of PA. The Leu72Met polymorphism did not have a significant effect on the change in PA during the intervention either.

The interaction between the −501A/C polymorphism of *GHRL* and PA on serum HDL cholesterol concentration is a novel finding. Physical activity increases HDL cholesterol levels, but there is large interindividual variability in the response [41]. Three studies have reported a positive correlation between plasma ghrelin and HDL cholesterol concentrations [42-44]. An association between the −1062 G/C polymorphism in the promoter of *GHRL* and serum HDL cholesterol concentration has also been reported [45]. It has been suggested that HDL particles have a role as circulating ghrelin transporters [46]. However, recent studies also indicate that ghrelin analogs may affect cholesterol metabolism through binding CD36 and GH secretagogue receptors on macrophages, leading to cholesterol efflux into the HDL reverse pathway [47]. Further studies are needed to

clarify the mechanisms behind the association of ghrelin with HDL cholesterol.

No interactions between polymorphism in *LEPR* and PA on blood pressure have been reported previously. Differences in the response to a 3-month lifestyle modification of caloric restriction and moderate PA among the genotypes of the Lys656Asn polymorphism in *LEPR* have, however, been reported [48]. Systolic blood pressure decreased significantly in the Lys656 homozygotes, but not in the carriers of the Asn656 allele [48]. Moreover, although the Lys109Arg polymorphism has not been reported to modify the effect of PA on blood pressure, it modified the effect of a 20-week endurance training program on measures of glucose homeostasis in the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study [28].

Leptin is an adipocyte-secreted hormone that is mainly involved in regulating energy homeostasis [49], but may also affect blood pressure by stimulating sympathetic outflow [50,51]. High circulating levels of leptin may partly explain the increase in renal sympathetic tone observed in obese people [52]. The DNA sequence variation in the *LEPR* gene may impair the effect of leptin on the receptor and thus attenuate the favorable effects of PA on blood pressure. Exercise training alone or in combination with dietary modification may also decrease serum leptin levels independent of weight loss [53,54].

Although our study was carried out according to well-controlled trial design, it has some limitations regarding current analyses. Statistically, the sample size was rather small for interaction analyses and mostly too small to evaluate effects of additive and recessive genotypic models. Moreover, because multiple statistical tests were performed, the results may represent false positives and should be confirmed in other large-scale studies. Ultimately, a specific randomized clinical trial would be required to provide a definitive answer on whether polymorphisms in *GHRL* and *LEPR* genes modify the effects of PA on changes in features of the metabolic syndrome.

As the DPS was designed to assess the combined effect of increased PA, dietary modification, and weight reduction on the risk of developing T2D, it did not include a separate exercise group. Therefore, we cannot completely rule out the effects of dietary changes or weight loss on our results, although we adjusted the analyses for diet and weight. Moreover, accurate assessment of habitual PA itself is problematic in epidemiologic studies. Although PA questionnaires are cost-effective and applicable for large study populations, they are also subject to recall bias. The correlation between vigorous PA measured with the Kuopio Ischemic Heart Disease Risk Factor Study questionnaire and VO_2max is, however, quite strong ($r = 0.40$) [55]. The questionnaire has also been found to be quite repeatable [22]. In addition, the annual administration of the questionnaire and the use of averaged PA levels reduce the measurement variability.

In summary, changes in PA did not significantly modify the associations of polymorphisms in the *ADRB2*, *ADRB3*, *TNF*, *IL6*, *IGF1R*, *LIPC*, *LEPR*, or *GHRL* genes with the conversion from IGT to T2D. However, the Leu72Met (rs696217) polymorphism in *GHRL* modified the effect of moderate-to-vigorous PA on the changes in body weight and waist circumference, the −501A/C (rs26802) polymorphism in *GHRL* modified the effect of total and moderate-to-vigorous PA on the change in HDL cholesterol, and the Lys109Arg (rs1137100) polymorphism in *LEPR* modified the effect of total PA on the change in systolic blood pressure.

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