Analyses of single nucleotide polymorphisms in selected nutrient-sensitive genes in weight-regain prevention: the DIOGENES study¹⁻⁴

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ABSTRACT
Background: Differences in the interindividual response to dietary intervention could be modified by genetic variation in nutrient-sensitive genes.

Objective: This study examined single nucleotide polymorphisms (SNPs) in presumed nutrient-sensitive candidate genes for obesity and obesity-related diseases for main and dietary interaction effects on weight, waist circumference, and fat mass regain over 6 mo.

Design: In total, 742 participants who had lost ≥8% of their initial body weight were randomly assigned to follow 1 of 5 different ad libitum diets with different glycemic indexes and contents of dietary protein. The SNP main and SNP-diet interaction effects were analyzed by using linear regression models, corrected for multiple testing by using Bonferroni correction and evaluated by using quantile-quantile (Q-Q) plots.

Results: After correction for multiple testing, none of the SNPs were significantly associated with weight, waist circumference, or fat mass regain. Q-Q plots showed that ALOX5AP rs4769873 showed a higher observed than predicted P value for the association with less waist circumference regain over 6 mo (−3.1 cm/allele; 95% CI: −4.6, −1.6; P/Bonferroni-corrected P = 0.000039/0.076), independently of diet. Additional associations were identified by using Q-Q plots for SNPs in ALOX5AP, TNF, and KCNJ11 for main effects; in LPL and TUB for glycemic index interaction effects on waist circumference regain; in GHRL, CCK, MLXIPL, and LEPR on weight; in PPARCA, PCK2, ALOX5AP, PYY, and ADRB3 on waist circumference; and in PPARD, FABP1, PLAUR, and LPIN1 on fat mass regain for dietary protein interaction.

Conclusion: The observed effects of SNP-diet interactions on weight, waist, and fat mass regain suggest that genetic variation in nutrient-sensitive genes can modify the response to diet. This trial was registered at clinicaltrials.gov as NCT00390637.

INTRODUCTION
Successful weight loss does not only include the weight-loss period and the obtained clinically significant weight loss of 5% to 10%; the maintenance period after the weight loss is pivotal in preventing weight regain and repeated cycles of weight loss and weight regain. Studies have shown that only 20% of US adults are successful in preventing weight regain after a clinically significant weight loss (1, 2).

Several dietary approaches have been investigated with regard to weight loss and maintenance (3). An attractive solution to prevent weight regain is to provide a diet with a macronutrient composition that is satiating at a low caloric density, eg, a diet with a high dietary protein content that increases satiety and decreases ad libitum energy intake compared with diets high in carbohydrate or fat (3) or a diet with a low glycemic index (LGI)⁵ that has been shown to increase weight loss and delay weight regain, even when offered ad libitum (4).

The Diet, Obesity and Genes (DIOGENES) study showed that an ad libitum diet high in dietary protein and with an LGI is significantly better at preventing weight regain over 6 mo than are diets with a high glycemic index (HGI) and low dietary protein (LP) or...
high dietary protein (HP), with an LGI and LP, or with the official country-specific diet recommendations (5). The results also showed that an HP diet, compared with an LP diet (difference of 5.4% of energy; \( P < 0.001 \)), produced a smaller weight regain (0.93 kg, 0.31–1.55; \( P = 0.003 \)) and that an LGI diet, compared with an HGI diet (5–GI unit difference; \( P < 0.001 \)), produced a smaller weight regain (0.95 kg, 0.33–1.57; \( P = 0.003 \)).

There were, however, large interindividual differences in weight regain within the diet groups, and it could be possible that these differences are dependent on genetic variation in nutrient-sensitive genes (6). The aim of this study was to examine whether single nucleotide polymorphisms (SNPs) in presumed nutrient-sensitive genes, by main effects or by interaction effects with dietary protein or GI, were associated with weight, waist circumference, or fat mass regain during a 6-mo ad libitum weight-maintenance diet.

**SUBJECTS AND METHODS**

**Study design**

The DIOGENES study is a randomized, controlled 6-mo dietary intervention that examines the effects of dietary protein and GI on weight regain and metabolic and cardiovascular risk factors in overweight and obese families, after an 8-wk weight loss period on a low-calorie diet (LCD), in 8 research centers in Europe (5,7–9). The study included families with at least one loss period on a low-calorie diet (LCD), in 8 research centers in Europe (5,7–9). The study included families with at least one overweight or obese [27 ≥ BMI (in kg/m²) ≤ 45] parent between 18 and 65 y and at least one healthy child between 5 and 17 y. Adult participants who achieved a weight loss ≥8% of their initial body weight were randomly assigned to 1 of 5 different ad libitum diets: 1) LP/LGI, 2) LP/HGI, 3) HP/LGI, 4) HP/HGI, or 5) control diet (5). The control diet was designed to follow the current official dietary guidelines for each of the countries. The study was approved by the local ethical committees in the respective countries, confirming that the study protocol was in accordance with the Declaration of Helsinki.

For the current analyses, a 2 × 2 factorial design was used to compare the LP with the HP group or the LGI with the HGI group for adults who lost ≥8% of their initial body weight, who were randomly assigned to the dietary intervention, and who were successfully genotyped.

**Selection of candidate genes and tagSNPs**

The candidate genes were selected on the basis of prior knowledge of whether the pathway, gene, gene transcript, or SNP was implicated in obesity, weight loss, weight regain, or associated diseases with emphasis on interaction with dietary protein or GI, from the literature, or the IntegraGen database. For the presumed nutrient-sensitive candidate genes, a comprehensive approach was used to ensure genetic coverage of the locus (±5 kilobases) by selecting tagSNPs for each of the selected genes. The tagSNPs were identified from the International HapMap data for European ancestry (release 20, NCBI build 35), and the LD (linkage disequilibrium) structure was evaluated by using Haploview software, version 3.32 (10). TagSNPs were selected by using Tagger (11) with single-marker option with an LD threshold of \( r^2 = 0.8 \), except for the genes APP, TUB, KCNJ11, ABCC8, and PPARGC1A for which tagSNPs were selected with an LD threshold of \( r^2 = 0.7 \). SNPs located in exonic regions, frequently studied, or included in the Illumina HumanHap 300 were preferentially included as tagSNPs, whereas SNPs with an expected low genotyping success rate [in close proximity to another SNP (60 base pairs) or in a repeat region] were deselected. In total, 768 tagSNPs were selected for genotyping. The candidate gene and tagSNP selection are described in more detail elsewhere (12).

**DNA extraction and genotyping**

For genetic analyses, DNA was extracted from EDTA-blood buffy coats stored at −80°C by KBioscience. Genotyping of all samples was performed by using the Illumina Bead Station System (Illumina Inc) by IntegraGen. Two Centre d’Etude du Poly morphisme Humain control samples were added on each plate: one was different on each plate and one was identical among the 15 genotyped plates. The reproducibility was 100% and the concordance rate was 99.6%. In total, 651 SNPs (in 69 genes) had a call rate ≥95% and a minor allele frequency >1%, and they did not deviate significantly (\( P > 0.001 \)) from Hardy-Weinberg equilibrium. All genotype analyses were performed and reported with respect to the minor allele (<50%), because the functional effects of all the selected SNPs are not known. A complete list of candidate genes and tagSNPs is given elsewhere (see Supplementary Table 1 under “Supplemental data” in the online issue).

**Statistical analyses**

The SNP-diet analyses were performed for interaction with either dietary protein, LP compared with HP, or GI, LGI compared with HGI, with respect to weight, waist circumference, and fat mass regain (outcomes) during the 6-mo (182 d) ad libitum diet. The initial diet variable with 5 levels (5 different diets) was recoded into 3 indicator variables, accounting for HP/LP, HGI/ LGI, and control diets. The LP and LGI groups were used as reference for the SNP-dietary protein and SNP-GI interaction analyses, respectively. The control diet analyses were omitted because the dietary advice varied between countries. Only the adults from the DIOGENES study were included in the analyses, and family structure, accounting for shared current environment, was defined as single-parent (10% of the individuals), 2-parent with one parent participating (55%), or 2-parent with 2 parents participating (35%). The statistical analyses were based on linear regressions founded in the following model formulation:

\[
O = I + t + t^2 + SNP \times DIET + SNP + DIET + SEX + FS + P + Age + BMI + LCDloss + e
\]

where \( O \) is outcome, \( I \) is intercept, \( t \) is time, \( t^2 \) is time squared; \( SNP \times DIET \) is the SNP-diet interaction effect, \( SNP \) is the SNP main effects, \( DIET \) is the diet main effect, \( SEX \) is the individual’s sex, \( FS \) is family structure, \( P \) is partner (clinical center), \( Age \) is baseline age, \( BMI \) is baseline BMI, \( LCD \) is weight loss during LCD, and \( e \) is the error term (assumed to be normally distributed). For the weight analyses, multiple available measurements during the maintenance diet were used [at a maximum of 9 time points (number of observations) per individual, and longitudinal models were fitted allowing within individual-correlations. The fitting procedure was quasi-least squares, which is based on the generalized
estimating equations approach, as implemented in Stata through
the xtqls-function. Covariance matrices were fitted based on
the Markovian structure, and robust SEs were estimated, which also
led to that all nonimputed covariates were updated to be linearly
dependent on time to facilitate sensible interpretations. The longi-
tudinal adjustment for weight response also partially accounts, as
a side effect of the increased underlying data set, for possible
bias caused by different dropout rates. For the fat mass and waist
circumference outcomes, standard linear regression analyses
were performed with additional adjustment for respective base-
line measure. Significance testing was performed and derived
with respect to the SNP main effects, and SNP-diet interaction
effect variables and corresponding quantile-quantile (Q-Q) plots
were created. Corrections for multiple testing were performed by
using Bonferroni correction for a total of 1953 tests (651
SNPs for SNP-main, SNP-GI, and SNP-dietary protein) per out-
come and the Benjamini-Hochberg false discovery rate. Q-Q
plots were used to graphically evaluate the results of the SNP
main and interaction effects. If the Q-Q plots showed lower
P values than expected from the null distribution, it was inter-
preted as possible true associations. All analyses were per-
formed by using Stata 9.2 (StataCorp LP).

RESULTS

The characteristics of the participants are summarized in
Table 1. The list of selected nutrient-sensitive candidate genes
and tagSNPs are given elsewhere (see Supplementary Table 1
under “Supplemental data” in the online issue). All results of the
main effects of SNPs, interaction effects between SNPs and
dietary protein, and interaction effects between SNPs and GI on
weight, waist circumference, and fat mass regain, respectively,
are given elsewhere (see Supplementary Tables 2–10 under
“Supplemental data” in the online issue).

After correction for multiple testing, none of the SNPs were
significantly associated with weight, waist circumference, or fat
mass regain either by main effects or interaction effects. The Q-Q
plots indicated that effects observed for SNP-dietary protein
interactions on weight regain, SNP main effects and SNP-dietary
protein and SNP-GI interaction effects on waist circumference
regain, and SNP-dietary protein interaction effects on fat mass
regain could be true associations (see Supplementary Figures 1–3
under “Supplemental data” in the online issue) despite being
nonsignificant after correction for multiple testing by using ei-
ther Bonferroni or Benjamini-Hochberg false discovery rate (see
Supplementary Tables 2–10 under “Supplemental data” in the
online issue). These SNPs included SNPs in ALOX5AP
rs4769873, rs9578196, and rs9315051; TNF rs1041981; and
KCNJ11+ABC8 rs2074308, which showed main effects on
waist circumference regain (Table 2). The pairwise LDs be-
 tween the ALOX5AP SNPs are 0.73 for rs4769873+rs9578196,
0.69 for rs4769873+rs9315051, and 0.49 for rs9578196
+rs9315051.

The SNPs that showed effects of interaction with GI or protein
on waist circumference regain were in LPL and TUB and
PPARGC1A, PCK2, ALOX5AP, ADRB3, and PYY (Table 2).
The pairwise LDs between the PPARGC1A SNPs are 0.48 for
rs2970848+rs2932976, 0.16 for rs2970848+rs2970853, and 0.07
rs2932976+rs2970853. The pairwise LD between the ALOX5AP
SNPs is 0.54 for rs4076128+rs10507391. In terms of an in-
creased risk of waist circumference regain of 6.3 cm/6 mo (95% CI: 2.4, 10.2), compared with
noncarriers.

The SNPs that showed an effect on weight regain in interaction
dietary protein were SNPs located in or close to
GHRL, CCK, MLXIPL, and LEPR (Figure 1), based on Q-Q plots (see
Supplementary Figure 1 under “Supplemental data” in the
online issue). The pairwise LD between the LEPR SNPs is 0.03.
Allele loading of the combination of GHRL rs17032621
AG/AA, PCK2 rs11629199 GG, and ALOX5AP
rs10507391 CC was observed in 9% of the participants, and this
combination of alleles led to an increased waist circumference
regain of 6.3 cm/6 mo (95% CI: 2.4, 10.2), compared with
noncarriers.

Table 1

| Participant characteristics at baseline, after the 8-wk LCD weight-loss period, and after the 6-mo weight-maintenance period |
|--------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| | Low dietary protein & n | High dietary protein & n | Control diet & n |
| Baseline | | | | | | |
| Age (y) | 146 41 ± 6 150 41 ± 6 | 155 42 ± 7 146 41 ± 6 | 145 42 ± 7 |
| Sex (% F) | 146 65 150 67 | 155 66 146 60 | 145 65 |
| Weight (kg) | 146 89.4 ± 15.8 150 88.4 ± 14.8 | 155 88.9 ± 15.6 146 89.5 ± 17.0 | 145 89.1 ± 15.7 |
| WC (cm) | 146 98 ± 12 150 98 ± 12 | 155 97 ± 12 146 97 ± 13 | 145 98 ± 13 |
| Fat mass (kg) | 146 32.4 ± 9.9 150 32.5 ± 10.4 | 155 32.5 ± 11.2 146 32.2 ± 10.4 | 145 32.3 ± 9.8 |
| After 8-wk LCD | | | | |
| Δ1Weight (kg) | 146 -11.2 ± 3.4 150 -11.0 ± 3.7 | 155 -11.0 ± 3.5 146 -11.0 ± 3.0 | 145 -11.1 ± 3.8 |
| Δ1WC (cm) | 142 -10.1 ± 4.5 145 -9.7 ± 4.7 | 150 -9.7 ± 4.4 144 -10.3 ± 4.6 | 141 -9.7 ± 5.0 |
| Δ1Fat mass (kg) | 116 -8.4 ± 5.2 110 -7.6 ± 5.0 | 111 -8.0 ± 5.2 104 -8.6 ± 5.3 | 107 -8.4 ± 4.2 |
| After 6-mo ad libitum diet | | | | |
| Δ2Weight (kg) | 106 0.3 ± 4.9 97 1.7 ± 5.2 | 124 -0.4 ± 6.5 107 0.6 ± 5.5 | 114 0.8 ± 4.8 |
| Δ2WC (cm) | 101 0.5 ± 7.1 91 0.9 ± 7.0 | 115 -0.3 ± 6.4 101 1.1 ± 6.5 | 105 0.7 ± 5.6 |
| Δ2Fat mass (kg) | 86 -1.0 ± 4.6 75 -0.1 ± 4.6 | 102 -1.0 ± 5.3 87 -0.2 ± 5.9 | 93 -0.8 ± 4.4 |

1 All values are means ± SDs. Age, sex, weight, WC, and fat mass were measured at baseline; Δ1, weight, WC, or fat mass loss from baseline to week 8 of a low-caloric diet (for the participants who were randomly assigned to the ad libitum 6-mo period); Δ2, weight, WC, or fat mass regain from randomization to 6 mo (182 d) of the diet. GI, glycemic index; LCD, low-calorie diet; WC, waist circumference.
in 8% of the participants and resulted in a mean estimated increased weight regain of 4.1 kg/6 mo (95% CI: 1.7, 6.7), compared with noncarriers.

The SNPs that showed an effect on fat mass regain in interaction with dietary protein comprised SNPs in $PPARD$, $FABP1$, $LPIN1$, and $PLAUR$ (Figure 2), based on Q-Q plots (see Supplementary Figure 3 under “Supplemental data” in the online issue). The pairwise LD between the $PPARD$ SNPs is 0.28. Allele loading of the combination of $PPARD$ rs6457816 AG/GG, $PPARD$ rs9658119 AC/CC, and $FABP1$ rs2970902 CC was observed for 5.0% of the participants and led to an increased fat mass regain of 5.0 kg/6 mo (95% CI: 1.1, 8.8), compared with noncarriers.

DISCUSSION

In this large-scale multicenter intervention study, the DIOGENES study, investigating the weight-maintenance properties of dietary protein and GI, we examined SNP main effects and SNP-diet interaction effects on weight, waist circumference, or fat mass regain for 651 tagSNPs, covering the genetic variation of 69 presumably nutrient-sensitive candidate genes.

Despite the selection of presumed nutrient-sensitive genes with complete coverage of genetic variation in the 69 loci, significant separation of the LP compared with the HP and GI groups, and careful monitoring of dietary intake and weight regain, none of the SNP-diet interaction effects remained significant after correction for multiple testing. However, comparisons of the observed $P$ values with the null distribution by Q-Q plots indicated that some of the observed associations were likely to be true associations.

The rs4769873 in the adipokine $ALOX5AP$, which has been linked to atherosclerosis and cardiovascular diseases, showed an association with waist circumference regain that was possibly a true association based on the Q-Q plots but was still not significant after correction for multiple testing. The rs4769873 was significant after correction for multiple testing. The rs4769873 was in moderate LD with the rs9578196 and rs9315051, and the SNPs might therefore represent an $ALOX5AP$ haplotype that interacts with dietary protein to modify abdominal obesity. $ALOX5AP$ transfers arachidonic acid to 5-lipogenase in the first step of the leukotriene biosynthesis in which one of the end products is the proinflammatory leukotriene B4 (13), a ligand for the PPARA (14) and inducer of the proinflammatory chemokine (C-C motif) ligand 2 (15). Previously, $ALOX5AP$ expression has been shown to be associated with obesity and insulin resistance; however, no associations between $ALOX5AP$ haplotypes and obesity were identified (16).

The selection of nutrient-sensitive genes was done by careful evaluation of evidence of nutrient sensitivity and relation to obesity or obesity-related diseases and as such is based on a hypothesis-driven candidate gene approach. Through this selection of genes, we introduced some limitations to our analyses, because it excluded the possibility of discovering new genes involved in regulating weight, waist circumference, or fat mass. Furthermore, by not including SNPs recently identified in genome-wide association studies, we also excluded the examination of the effects of these SNPs on weight regain. In that respect, it could be argued that a genome-wide association study approach should have been used to initially identify nutrient-sensitive SNPs that might not have been discovered previously. However, such a study would require a large population because of an expected small effect size (17), and the design of a gene-diet interaction study would be a compromise between a large population, precision in the measured phenotypes, and the control of other environmental factors (18).

Instead, the selected nutrient-sensitive genes tagSNPs were chosen...
to cover the genetic variance of the selected genes plus 500 base pairs up- and downstream from the coding region with an LD threshold of 0.7 to 0.8.

The DIOGENES study has a controlled exposure to the dietary components investigated with evaluation of food intake by repeated 3-d weighted food registration and nitrogen excretion (5).
as recommended for studies on gene-environment interactions (18). For dietary intervention studies of weight regain, significant differentiation between interventions can be difficult to obtain (19); however, the DIOGENES study did obtain a significant difference between the LP and HP and LGI and HGI diets. Also, the study includes several measurements of body weight during the 6-mo ad libitum diet period; thus, we can use longitudinal data that allows us to account for possible bias caused by different dropout rates for the diet and, therefore, the analyses of body weight regain can be regarded as the most robust data of the SNP main and SNP-diet interaction data presented.

Previous studies have investigated SNPs in single genes or a few selected genes in relation to weight loss and regain (19–22). In most of these studies, only the main effects of the SNPs are investigated (20) and not the gene-diet interaction effects. A recent article from the epidemiologic part of the DIOGENES study examined the SNP-diet interaction of 123 SNPs in 15 genes in the hypothalamic pathway in 6566 individuals in a case-cohort design and identified one association (\(P = 2 \times 10^{-7}\)) with weight regain for the interaction between neuromedin B rs7180849 and GI (12). However, we could not replicate this finding in the DIOGENES intervention study (\(P = 0.60\)).
In conclusion, the analyses of tagSNPs in the selected 69 candidate genes suggests that genetic variation in nutrient-sensitive genes can affect weight, waist circumference, or fat mass regain by interacting with dietary protein or GI. However, further studies are needed to verify the observed gene-diet interaction effects on regain outcomes and to identify new nutrient-sensitive genes.

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