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Variation at the Melanocortin 4 Receptor gene and response to weight-loss interventions in the Diabetes Prevention Program

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Abstract

Objective—To assess associations and genotype × treatment interactions for melanocortin 4 receptor (MC4R) locus variants and obesity-related traits.

Design and Methods—Diabetes Prevention Program (DPP) participants (N=3,819, of whom 3,356 were genotyped for baseline and 3,234 for longitudinal analyses) were randomized into intensive lifestyle modification (diet, exercise, weight loss), metformin or placebo control. Adiposity was assessed in a subgroup (n=909) using computed tomography. All analyses were adjusted for age, sex, ethnicity and treatment.

Results—The rs1943218 minor allele was nominally associated with short-term (6 month; \( P=0.032 \)) and long-term (2 year; \( P=0.038 \)) weight change. Eight SNPs modified response to treatment on short-term (rs17066856, rs9966412, rs17066859, rs8091237, rs17066866, rs7240064) or long-term (rs12970134, rs17066866) reduction in body weight, or diabetes incidence (rs17066829) (all \( P_{\text{interaction}} <0.05 \)).
**Conclusion**—This is the first study to comprehensively assess the role of $MC4R$ variants and weight regulation in a weight loss intervention trial. One $MC4R$ variant was directly associated with obesity-related traits or diabetes; numerous other variants appear to influence body weight and diabetes risk by modifying the protective effects of the DPP interventions.

**Keywords**

$MC4R$; Randomized Controlled Trial; Lifestyle; Metformin; Gene-Environment Interaction; Genetic; Obesity; Diabetes Prevention Program; Adiposity

**INTRODUCTION**

Obesity is a highly heritable(1), complex trait presumed to originate from interactions between multiple genetic and environment risk factors. Several well-defined genetic causes of monogenic obesity are known(2), as are multiple low-penetrance obesogenic variants that are prevalent in the general population(3).

One of the loci implicated in both monogenic and common forms of obesity is the melanocortin 4 receptor (encoded by $MC4R$). Mutations in $MC4R$ are the most frequent known genetic causes of congenital obesity(4) and a variant mapping to a region upstream of $MC4R$ is strongly associated with variations in BMI and adiposity in multiple populations(5). $MC4R$ variants have also been associated with energy balance behaviors(6, 7), blood pressure(8), dyslipidemia(9), stature(4), and type 2 diabetes(10).

Two important risk factors for obesity are physical inactivity and excessive caloric intake. Thus, studies focusing on the interactions between obesity-predisposing genes and therapies that influence body weight may help elucidate the molecular mechanisms underlying obesity and its consequences, such as cardiovascular disease, certain cancers, osteoarthritis, and type 2 diabetes. In this regard, lifestyle interventions focused on diet, exercise, and pharmacologic interventions that influence weight loss (e.g., metformin) are relevant.

The purpose of this study was to extend earlier studies that center on $MC4R$ variants and BMI by examining whether $MC4R$ variation per se is associated with obesity-related traits over time or type 2 diabetes incidence. We tagged the entire $MC4R$ region and examined whether associations with obesity-related traits are mediated by dietary preference or physical activity or modified by metformin treatment or lifestyle intervention. All analyses were performed within the Diabetes Prevention Program (DPP), a randomized clinical trial of intensive lifestyle modification, metformin or troglitazone therapy, or placebo intervention.

**METHODS and PROCEDURES**

**The Diabetes Prevention Program (DPP)**

The DPP was a multi-center randomized clinical trial. The study design and details of the preventive interventions have been described in detail previously(11-13). Briefly, non-diabetic, overweight persons ($n=3,234$) with raised fasting and post-challenge glucose levels were randomized to placebo, metformin (850 mg twice daily), troglitazone (400 mg daily), or a
program of intensive lifestyle modification (aimed at ~7% weight loss and ~150 minutes of physical activity per week). A further 585 participants were randomized to receive troglitazone treatment; owing to liver toxicity, this aspect of the DPP trial was terminated early and only the baseline results were used in these analyses(11). Thus, a total of 3,819 participants were included in the baseline analyses reported here, whereas 3,234 are included in the prospective analyses. The principal endpoint in the DPP was the development of diabetes by confirmed oral glucose tolerance testing (OGTT). Other phenotypes, such as changes in weight, waist circumference, abdominal fat area, lipids, insulin and glucose, were also collected. The primary focus of this report is on the obesity-related traits; the secondary focus is on diabetes incidence. Each of the 27 DPP centers obtained approval from their respective institutional review boards prior to initiation of the study protocol.

Consent for genetic analyses was obtained in 87.9% (n=3,356/3,819) of participants who were originally assigned to a treatment arm in the DPP. Of the participants studied in this report, by self-report 56.1% were white, 20.4% African American, 16.7% Hispanic, 4.4% Asian American and 2.5% American Indian. Concordant with the entire DPP, the participants’ mean age at enrollment was 51 (SD=11) years and mean body mass index (BMI) was 34 (SD=7) kg/m$^2$; (see Table 1).

**Quantification of energy intake, physical activity, and body composition**

In-person interviews were conducted to obtain data on calorie intake, percent of calories from fat and protein, absolute intake of fat and protein and food preference behaviors at baseline and 1 year later using the semi-quantitative DPP food frequency questionnaire (FFQ)(14). To evaluate food preference behaviors, we assessed the frequency of consumption of snacks, sweets and desserts, vegetables and fruits, expressed as servings per day. Occupational and leisure-time physical activity (LTPA) was assessed using the validated Modifiable Activity Questionnaire (MAQ) at baseline and annually thereafter(15). Weight and BMI were measured in all participants.

Computed tomography (CT) was performed at baseline in 909 randomly selected subjects DPP participants, among whom 718 underwent a repeated measure at 12-months post-randomization to quantify subcutaneous and visceral fat areas (at the level of the lumbar 2-3 disc space)(16) and gave consent for genetic analyses. Among the participants with both baseline and one-year CT measurements, 241 were from the intensive lifestyle group, 246 from the metformin group and 231 from the placebo group.

**Genotyping**

SNPs were selected through multimarker tagging of the $MC4R$ locus (as previously described(17)). Genotyping was performed by allele-specific primer extension of multiplex amplified products and detection with matrix-assisted laser desorption ionization-time of flight mass spectroscopy on a Sequenom iPLEX platform. The genotyping success rates for the SNPs studied here were >99%. All 20 SNPs were in Hardy-Weinberg equilibrium in all five ethnic groups ($P>0.05$). For each SNP, genomic location, alleles and their frequencies
are reported in Online Supplementary Table 1. Online Supplementary Figures 1a-e show the LD plots for these variants.

**Statistical analysis**

Analyses were performed using the SAS software (SAS Institute, Cary, NC). Weight loss and abdominal fat area follow-up data were normally distributed and linear regressions were employed for these outcomes. SNP associations with diabetes incidence were assessed with Cox proportional hazards models. Potential mediating effects of leisure time physical activity (LTPA) and energy intake were tested by comparing the SNP regression coefficients with and without adjustment for the potential mediators(17). All models were adjusted for genotype, intervention, baseline value of the dependent variable, age at randomization, sex, and self-reported ethnicity. Further adjustment for study center made no material difference to the results (data not shown) and is thus not included in the current models. To determine whether it was appropriate to combine data from different treatment and ethnicity groups, we tested genotype × treatment and genotype × ethnicity interactions. If these tests were statistically significant \( (P<0.05) \), we stratified by group; otherwise data were pooled and adjusted for treatment and/or ethnicity. For all variants, we analyzed additive genetic models. Two-sided \( P \)-values without multiplicity adjustment are reported.

**MC4R** is a strong candidate gene for obesity-related traits(4); thus, the prior probability that variants at this locus are associated with obesity traits is likely to be above the null. Moreover, although the DPP is the largest randomized controlled trial of intensive lifestyle modification, it is modestly powered for most of the tests performed here (see:(18)) for description of the statistical power characteristics of the DPP). Thus, correcting for multiple testing is likely to increase type 2 error (false-negative) rates. Nonetheless, we are conscious that many readers will be keen to compare the nominal test statistics with those corrected for multiple testing. After considering the linkage disequilibrium between the 20 SNPs, we determined that there are 17.46 independent SNP tests(19). Therefore, instead of the Bonferroni cutoff of 0.05, a cutoff of 0.05/17.46=0.003 is used to determine a conservative estimate of statistical significance for the results reported in this paper.

Exploratory analyses were performed to determine whether any of the statistically significant genetic effects detected in the main analyses were likely to be mediated by specific lifestyle factors. The following factors were considered potential intermediate factors (mediators) of the observed genetic effects: leisure time physical activity in MET-hrs per week (metabolic equivalents), total caloric intake (kcal/day), percent of calories from fat, percent of calories from protein, fat intake (g/day), protein intake (g/day), snacking (number of snacks/day), sweet and dessert (servings/day), vegetable consumption (servings/day) and fruit consumption (servings/day).

While testing each candidate mediator, two models were fit, one with the candidate mediator as a covariate and the other without. From each pair of models, the difference in the two SNP regression coefficients and the standard errors of these differences were calculated(18). In these analyses, a statistically significant non-zero difference represents a discrepancy between the marginal SNP effect estimate and the SNP effect estimate that is conditional on
the putative mediator, thus validating the intermediate function of the mediator from genotype to the outcome.

RESULTS

Participant characteristics

Participant characteristics for the DPP cohort used for genetic analyses are reported elsewhere(17). Table 1 reports anthropometric traits, energy intake, and physical activity at baseline, as well as the weight loss outcomes.

Table 2 shows results from the analysis focused on the entire MC4R region; the table shows regression coefficients (representing the average difference in the dependent variable for each additional copy of the minor allele) and P values for all 20 MC4R SNPs and baseline weight and BMI in DPP participants. After adjustment for age, sex, and self-reported ethnicity, no SNP was statistically associated with baseline weight or BMI.

SNP associations with change in weight or adiposity

Tables 3 and 4 list the overall and treatment specific genetic effects for the three major outcomes: short-term (baseline to 6 months) and long-term (baseline to 2 years) weight change, and diabetes incidence. The major ‘C’ allele at SNP rs1943218 was associated with less short-term (P=0.032) and long-term (P=0.038) weight loss. Seven SNPs modified response to treatment on short-term (rs17066856, rs9966412, rs17066859, rs8091237, rs17066866, rs7240064) or long-term (rs12970134, rs17066866) reduction in body weight. Among them, nominally statistically significant associations with weight change for rs7240064 in the placebo arm and rs9966412 and rs17066859 in the metformin arm were observed, with each SNP’s minor allele being associated with less short-term weight loss. The minor allele of rs17066866 was associated with less short-term (P=0.006) and less long-term (P=0.004) weight loss in the lifestyle intervention group, the latter of which approached the multiple-testing corrected significance threshold (P=0.003).

Associations with diabetes incidence

Table 4 shows the SNP association results for type 2 diabetes incidence. SNP rs17066829 showed nominal evidence of interaction with treatment for diabetes risk (Pinteraction<0.05); for example, each copy of the minor allele at SNP rs17066829 corresponded to a HR of 0.80 (95% CI: 0.64-1.00) in the lifestyle group, which contrasted the effect observed in the placebo group (HR=1.09; 95% CI: 0.92-1.29).

Examining dietary factors and physical activity as putative mediators of genetic effects on obesity traits

Potential mediators were examined for all SNP associations and interactions that were of at least nominal statistical significance (as shown in Tables 3 and 4). No significant differences between the SNP effects before or after the adjustment of each potential mediator were detected.
MC4R is a strong candidate gene for diabetes and obesity-related traits. The rs17782313 SNP, which is upstream of the MC4R locus, has for example been reliably associated with common obesity in a GWAS meta-analysis(5) and also in the DPP(17), but the MC4R region in general has been of continued interest in obesity research for many years(3,5,10,21), largely because of its established role in monogenic obesity(4). Hence, we examined common variation across MC4R to seek a more detailed understanding of the role the gene plays in obesity and diabetes, and to determine whether the apparent obesogenic effect of variation at this locus is modified by weight loss interventions in the DPP.

In analyses adjusted for multiple statistical comparisons, we found nominal evidence of association between the rs1943218 variant and short- (baseline to 6 months) and long- (baseline to two years) term weight loss. Interactions between metformin treatment and the rs9966412 and rs17066859 variants on short-term weight loss were also found. Moreover, we observed a nominally significant statistical interaction between the rs17066829 SNP and treatment on diabetes incidence, which is of potential interest, as a recent study also showed that this variant was associated with BMI(22).

MC4R is centrally expressed and binds the α, β and γ isoforms of melanocortin-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) with varying affinity, while Agouti and Agouti-related protein (AgRP) are endogenous MC4R antagonists(23). Mc4r knockout mice are hyperphagic and are prone to maturity-onset obesity(24). Although restricting food availability reduces body weight in female Mc4r-/- mice, these animals remain susceptible to obesity(25), indicating that MC4R ablation also influences energy expenditure; direct evidence for this comes from observations that Mc4r null mice have lower resting oxidative energy metabolism(25) and dark-phase locomotor activity(25,26) compared with wild type animals. People heterozygous for specific MC4R mutations are MC4R deficient and are often hyperphagic, heavier and taller, with more fat and lean mass, and have higher bone mineral content than people who do not carry these mutations(27). Genome-wide scans of White Europeans have identified two independent signals for BMI at the MC4R locus, which are also associated with adipose mass and type 2 diabetes(10). One of the most thorough investigations of the MC4R locus in humans to date was undertaken by Thearle et al in a study of 6,760 persons of predominantly Pima Indian ancestry(28). The authors sequenced the MC4R exon to discover six low frequency mutations (~2.4%), which were subsequently functionally interrogated in vitro. These mutations conveyed an effect on weight gain but this was only evident in childhood, and the effect on type 2 diabetes that the authors observed was mediated by adult BMI.

MC4R is a strong candidate gene for obesity-related traits; thus, the prior probability that variants at this locus are associated with obesity traits is likely to be above the null, in which case the nominal P-values we report here may be somewhat conservative estimates of the underlying probability that an association exists. Moreover, although the DPP is the largest randomized controlled trial of intensive lifestyle modification, it is modestly powered for most of the tests performed here(17) for description of the statistical power characteristics of the DPP). Thus, correcting for multiple testing is likely to increase type 2 error (false-
negative) rates. Nonetheless, we are conscious that many readers will be keen to compare the nominal test statistics with those corrected for multiple testing, which is why the multiple-test corrected significance threshold is given above.

Assessing epidemiological observations in an experimental setting is an important step in the process of translating such observations into preventive action. Nevertheless, it is important to bear in mind that DPP participants were recruited on the basis of being overweight or obese and having impaired fasting and post-challenge glucose. Thus, the findings from this study may not generalize to other population subgroups. We were also unable to identify behavioral factors that might mediate the effects of MC4R on obesity-related traits, which would have aided the interpretation of the results described above. Our study was also limited by the absence of information on lifestyle behaviors after 12 months of intervention, which may have hampered the detection of mediators.

In summary, we have conducted the first comprehensive analysis of common genomic variation at the MC4R locus in relation to changes in body composition and incidence of type 2 diabetes within a randomized controlled trial of weight loss interventions. Our findings, that several MC4R variants are nominally related to these traits, in some instances by modifying the effects of weight loss interventions on diabetes incidence and adipose accumulation, adds novel information on the role of the MC4R locus in the development of obesity and type 2 diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Peter H. Bennett, MB, FRCP
Mary Beth Benson, RN, BSN
Evelyn C. Bird, RD, MPH
Brenda A. Broussard, RD, MPH, MBA, CDE
Marcella Chavez, RN, AS
Tara Dacawyma
Matthew S. Doughty, MD
Roberta Duncan, RD
Cyndy Edgerton, RD
Jacqueline M. Ghahate
Justin Glass, MD
Martia Glass, MD
Dorothy Gohdes, MD
Wendy Grant, MD
Robert L. Hanson, MD, MPH
Ellie Horse
Louise E. Ingraham, MS, RD, LN
Merry Jackson
Priscilla Jay
Roylen S. Kaskalla
David Kessler, MD
Kathleen M. Kobus, RNC-ANP
Jonathan Krakoff, MD
Catherine Manus, LPN
Sara Michaels, MD
Tina Morgan
Yolanda Nashboo (deceased)
Julie A. Nelson, RD
Steven Poirier, MD
Evette Polczynski, MD
Mike Reidy, MD
Jeanine Roumain, MD, MPH
Debra Rowse, MD
Sandra Sangster
Janet Sewenemewa
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Solome Abebe
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Yuping Gao
Kristina L. Grimes
Nisha Grover
Lori Haffner, MS
Steve Jones
Tara L. Jones
Richard Katz, MD
John M. Lachin, ScD
Pamela Mucik
Robert Orlosky
James Rochon, PhD
Alla Sapozhnikova
Hanna Sherif, MS
Charlotte Stimpson
Marinella Temprosa, MS
Fredricka Walker-Murray

Central Biochemistry Laboratory (Seattle, WA)
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F. Alan Aldrich

Carotid Ultrasound
Dan O’Leary, MD*

CT Scan Reading Center
Elizabeth Stamm, MD*

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Ronald J. Prineas, MD, PhD**/
Teresa Alexander
Charles Campbell, MS
Sharon Hall
Yabing Li, MD
Margaret Mills
Nancy Pemberton, MS
Farida Rautaharju, PhD
Zhuming Zhang, MD

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Elizabeth Mayer-Davis, PhD*
Robert R. Moran, PhD**

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Sanford Garfield, PhD

**Centers for Disease Control & Prevention (Atlanta, GA)**
Edward Gregg, PhD
Ping Zhang, PhD

**University of Michigan (Ann Arbor, MI)**
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Kathleen Jablonski, PhD5
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References


Obesity (Silver Spring). Author manuscript; available in PMC 2014 May 16.


What is already known about this subject

- *MC4R* encodes one of five melanocortin receptors that control energy balance, adipose deposition, and other metabolic traits
- *MC4R* mutations are the most frequent known cause of human monogenic obesity
- Common variants in *MC4R* are associated with common obesity and type 2 diabetes

What this study adds

- This study provides the first a comprehensive assessment of *MC4R* variation in relation to dynamic body weight phenotypes in a randomized controlled trial of weight loss interventions
- Several *MC4R* variants appear to modify the effects of the DPP weight loss interventions on reductions in body weight and diabetes incidence
Table 1

Characteristics of DPP study participants included in these analyses (N=3,891)

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>Lifestyle</th>
<th>Metformin</th>
<th>Placebo</th>
<th>Troglitazonea</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1079</td>
<td>1073</td>
<td>1082</td>
<td>585</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32%</td>
<td>34%</td>
<td>31%</td>
<td>34%</td>
<td>0.404</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51 (11)</td>
<td>51 (10)</td>
<td>50 (10)</td>
<td>50 (10)</td>
<td>0.469</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94 (21)</td>
<td>94 (20)</td>
<td>94 (20)</td>
<td>93 (19)</td>
<td>0.774</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34 (7)</td>
<td>34 (7)</td>
<td>34 (7)</td>
<td>34 (6)</td>
<td>0.311</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>105 (15)</td>
<td>105 (14)</td>
<td>105 (14)</td>
<td>104 (14)</td>
<td>0.605</td>
</tr>
<tr>
<td>SAT L2-3 (cm²)</td>
<td>308 (125)</td>
<td>301 (129)</td>
<td>314 (127)</td>
<td></td>
<td>0.449</td>
</tr>
<tr>
<td>SAT L4-5 (cm²)</td>
<td>443 (152)</td>
<td>433 (154)</td>
<td>449 (151)</td>
<td></td>
<td>0.408</td>
</tr>
<tr>
<td>VAT L2-3 (cm²)</td>
<td>200 (85)</td>
<td>195 (83)</td>
<td>199 (90)</td>
<td></td>
<td>0.701</td>
</tr>
<tr>
<td>VAT L4-5 (cm²)</td>
<td>163 (65)</td>
<td>158 (65)</td>
<td>159 (65)</td>
<td></td>
<td>0.500</td>
</tr>
<tr>
<td>LTPA (MET-hrs/wk)</td>
<td>16 (22)</td>
<td>16 (26)</td>
<td>17 (29)</td>
<td>15 (16)</td>
<td>0.390</td>
</tr>
<tr>
<td>Energy Intake (kcal/day)</td>
<td>2137 (1071)</td>
<td>2144 (986)</td>
<td>2098 (1052)</td>
<td>2118 (989)</td>
<td>0.739</td>
</tr>
<tr>
<td>Short-term (6m) weight loss (kg)</td>
<td>6.7 (5.6)</td>
<td>2.3 (4.0)</td>
<td>0.3 (4.0)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Long-term (2y) weight loss (kg)</td>
<td>5.4 (7.6)</td>
<td>2.1 (5.7)</td>
<td>0.0 (5.5)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Diabetes Incidence per 1,000 p-yr</td>
<td>50</td>
<td>80</td>
<td>100</td>
<td>74</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are percentage or Mean (SD), stratified by treatment assignment. P-values from ANOVA F-tests and Chi-Square test of independence. LTPA: leisure-time physical activity. The sample size (N=3,819) is the maximum number used in baseline analyses and includes participants who were subsequently randomized to troglitazone treatment, in addition to those randomized to lifestyle, metformin, or placebo. Sample sizes for: short-term weight loss (n=3085); long-term weight loss (n=3015); diabetes risk (n=3234). We include individuals subsequently randomized to troglitazone in the baseline analyses to maximize statistical power. However, these individuals are not included in the prospective analyses as they represent a relatively small subgroup of the DPP population, which is likely underpowered for treatment stratified analyses.

aThe troglitazone arm was terminated early(11) and, by consequence, only baseline data are used in this paper.
Table 2

Association between MC4R SNPs with baseline weight (kg) and BMI (kg/m$^2$) (N=3,346–3,356)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor(major) allele</th>
<th>Weight (kg/allele)</th>
<th>Coefficient (SE)</th>
<th>P-value</th>
<th>BMI (kg/m$^2$/allele)</th>
<th>Coefficient (SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1943226</td>
<td>G(T)</td>
<td>0.29 (0.94)</td>
<td>0.759</td>
<td>0.759</td>
<td>-0.19 (0.32)</td>
<td>0.556</td>
<td></td>
</tr>
<tr>
<td>rs11875096</td>
<td>C(T)</td>
<td>0.80 (1.21)</td>
<td>0.509</td>
<td>0.766</td>
<td>0.12 (0.80)</td>
<td>0.790</td>
<td></td>
</tr>
<tr>
<td>rs1943224</td>
<td>G(A)</td>
<td>-1.08 (2.36)</td>
<td>0.648</td>
<td>0.556</td>
<td>0.21 (0.80)</td>
<td>0.790</td>
<td></td>
</tr>
<tr>
<td>rs7255242</td>
<td>C(A)</td>
<td>0.92 (2.06)</td>
<td>0.654</td>
<td>0.882</td>
<td>-0.10 (0.69)</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>rs11872992</td>
<td>A(G)</td>
<td>-0.19 (0.73)</td>
<td>0.791</td>
<td>0.992</td>
<td>-0.00 (0.25)</td>
<td>0.992</td>
<td></td>
</tr>
<tr>
<td>rs8093815</td>
<td>T(C)</td>
<td>0.10 (0.52)</td>
<td>0.848</td>
<td>0.955</td>
<td>-0.01 (0.17)</td>
<td>0.955</td>
<td></td>
</tr>
<tr>
<td>rs17066856</td>
<td>C(T)</td>
<td>0.11 (0.67)</td>
<td>0.874</td>
<td>0.611</td>
<td>0.11 (0.23)</td>
<td>0.611</td>
<td></td>
</tr>
<tr>
<td>rs17066836</td>
<td>G(C)</td>
<td>0.33 (1.41)</td>
<td>0.815</td>
<td>0.876</td>
<td>0.07 (0.47)</td>
<td>0.876</td>
<td></td>
</tr>
<tr>
<td>rs1943227</td>
<td>G(A)</td>
<td>-0.57 (0.80)</td>
<td>0.480</td>
<td>0.747</td>
<td>-0.09 (0.27)</td>
<td>0.747</td>
<td></td>
</tr>
<tr>
<td>rs1943218</td>
<td>C(T)</td>
<td>-0.18 (0.54)</td>
<td>0.732</td>
<td>0.651</td>
<td>-0.08 (0.18)</td>
<td>0.651</td>
<td></td>
</tr>
<tr>
<td>rs17066829</td>
<td>A(T)</td>
<td>-0.33 (0.51)</td>
<td>0.510</td>
<td>0.340</td>
<td>-0.16 (0.17)</td>
<td>0.340</td>
<td></td>
</tr>
<tr>
<td>rs9966412</td>
<td>A(G)</td>
<td>0.88 (0.64)</td>
<td>0.168</td>
<td>0.102</td>
<td>0.35 (0.21)</td>
<td>0.102</td>
<td></td>
</tr>
<tr>
<td>rs17066859</td>
<td>A(G)</td>
<td>0.44 (0.76)</td>
<td>0.568</td>
<td>0.351</td>
<td>0.24 (0.26)</td>
<td>0.351</td>
<td></td>
</tr>
<tr>
<td>rs9965495</td>
<td>T(C)</td>
<td>-0.01 (0.52)</td>
<td>0.983</td>
<td>0.890</td>
<td>-0.02 (0.18)</td>
<td>0.890</td>
<td></td>
</tr>
<tr>
<td>rs12970134</td>
<td>A(G)</td>
<td>0.59 (0.59)</td>
<td>0.316</td>
<td>0.509</td>
<td>0.13 (0.20)</td>
<td>0.509</td>
<td></td>
</tr>
<tr>
<td>rs17700633</td>
<td>T(C)</td>
<td>0.40 (0.50)</td>
<td>0.425</td>
<td>0.989</td>
<td>0.00 (0.19)</td>
<td>0.989</td>
<td></td>
</tr>
<tr>
<td>rs1873305</td>
<td>G(T)</td>
<td>-0.29 (0.93)</td>
<td>0.752</td>
<td>0.805</td>
<td>-0.08 (0.31)</td>
<td>0.805</td>
<td></td>
</tr>
<tr>
<td>rs8091237</td>
<td>G(C)</td>
<td>0.12 (0.64)</td>
<td>0.854</td>
<td>0.510</td>
<td>-0.14 (0.22)</td>
<td>0.510</td>
<td></td>
</tr>
<tr>
<td>rs17066866</td>
<td>T(A)</td>
<td>0.52 (1.09)</td>
<td>0.633</td>
<td>0.429</td>
<td>0.29 (0.37)</td>
<td>0.429</td>
<td></td>
</tr>
<tr>
<td>rs7240064</td>
<td>T(C)</td>
<td>-0.14 (0.70)</td>
<td>0.838</td>
<td>0.526</td>
<td>-0.15 (0.24)</td>
<td>0.526</td>
<td></td>
</tr>
</tbody>
</table>

Coefficients, Standard Errors (SE) and P-values correspond to the additive allele effects adjusted for age at randomization, sex and ethnicity. The reported P-values are not adjusted for multiple comparison.
Table 3

SNP effects on short-term, long-term weight loss and diabetes risk

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor (major) allele</th>
<th>Coefficient (SE)</th>
<th>P-value</th>
<th>Coefficient (SE)</th>
<th>P-value</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1943226</td>
<td>G(T)</td>
<td>0.35 (0.24)</td>
<td>0.144</td>
<td>0.10 (0.33)</td>
<td>0.774</td>
<td>1.00 (0.91-1.11)</td>
<td>0.980</td>
</tr>
<tr>
<td>rs11875096</td>
<td>G(T)</td>
<td>0.06 (0.31)</td>
<td>0.835</td>
<td>0.30 (0.42)</td>
<td>0.473</td>
<td>0.97 (0.77-1.23)</td>
<td>0.805</td>
</tr>
<tr>
<td>rs1943224</td>
<td>G(A)</td>
<td>0.02 (0.59)</td>
<td>0.968</td>
<td>0.30 (0.82)</td>
<td>0.715</td>
<td>1.18 (0.78-1.79)</td>
<td>0.431</td>
</tr>
<tr>
<td>rs7235242</td>
<td>C(A)</td>
<td>0.06 (0.53)</td>
<td>0.908</td>
<td>-0.07 (0.73)</td>
<td>0.920</td>
<td>1.28 (0.88-1.86)</td>
<td>0.194</td>
</tr>
<tr>
<td>rs11872992</td>
<td>A(G)</td>
<td>-0.11 (0.18)</td>
<td>0.566</td>
<td>-0.18 (0.26)</td>
<td>0.474</td>
<td>1.04 (0.90-1.20)</td>
<td>0.606</td>
</tr>
<tr>
<td>rs8093815</td>
<td>T(C)</td>
<td>0.13 (0.13)</td>
<td>0.304</td>
<td>0.32 (0.18)</td>
<td>0.081</td>
<td>0.99 (0.90-1.09)</td>
<td>0.854</td>
</tr>
<tr>
<td>rs17066856</td>
<td>C(T)</td>
<td>-</td>
<td>-</td>
<td>0.20 (0.24)</td>
<td>0.392</td>
<td>0.94 (0.83-1.07)</td>
<td>0.375</td>
</tr>
<tr>
<td>rs17066836</td>
<td>G(C)</td>
<td>-0.12 (0.36)</td>
<td>0.734</td>
<td>-0.10 (0.49)</td>
<td>0.834</td>
<td>0.84 (0.63-1.11)</td>
<td>0.216</td>
</tr>
<tr>
<td>rs1943227</td>
<td>G(A)</td>
<td>0.13 (0.20)</td>
<td>0.507</td>
<td>0.05 (0.28)</td>
<td>0.847</td>
<td>0.95 (0.81-1.12)</td>
<td>0.540</td>
</tr>
<tr>
<td>rs1943218</td>
<td>C(T)</td>
<td>0.29 (0.14)</td>
<td>0.032</td>
<td>0.39 (0.19)</td>
<td>0.038</td>
<td>0.99 (0.89-1.10)</td>
<td>0.869</td>
</tr>
<tr>
<td>rs17066829</td>
<td>A(T)</td>
<td>0.02 (0.13)</td>
<td>0.884</td>
<td>0.14 (0.18)</td>
<td>0.420</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rs9966412</td>
<td>A(G)</td>
<td>-</td>
<td>-</td>
<td>0.25 (0.23)</td>
<td>0.260</td>
<td>0.98 (0.86-1.11)</td>
<td>0.737</td>
</tr>
<tr>
<td>rs7066859</td>
<td>A(G)</td>
<td>-</td>
<td>-</td>
<td>0.44 (0.27)</td>
<td>0.103</td>
<td>0.94 (0.81-1.09)</td>
<td>0.403</td>
</tr>
<tr>
<td>rs9965495</td>
<td>T(C)</td>
<td>0.17 (0.13)</td>
<td>0.207</td>
<td>0.20 (0.18)</td>
<td>0.265</td>
<td>1.00 (0.91-1.11)</td>
<td>0.980</td>
</tr>
<tr>
<td>rs12970134</td>
<td>A(G)</td>
<td>-0.09 (0.15)</td>
<td>0.548</td>
<td>-</td>
<td>-</td>
<td>1.02 (0.91-1.14)</td>
<td>0.788</td>
</tr>
<tr>
<td>rs17700633</td>
<td>T(C)</td>
<td>0.15 (0.14)</td>
<td>0.273</td>
<td>0.10 (0.20)</td>
<td>0.596</td>
<td>0.97 (0.87-1.08)</td>
<td>0.595</td>
</tr>
<tr>
<td>rs1873305</td>
<td>G(T)</td>
<td>-0.08 (0.24)</td>
<td>0.738</td>
<td>-0.18 (0.32)</td>
<td>0.577</td>
<td>0.99 (0.83-1.18)</td>
<td>0.903</td>
</tr>
<tr>
<td>rs8091237</td>
<td>G(C)</td>
<td>-</td>
<td>-</td>
<td>0.15 (0.23)</td>
<td>0.521</td>
<td>0.99 (0.87-1.12)</td>
<td>0.815</td>
</tr>
<tr>
<td>rs7066866</td>
<td>T(A)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.98 (0.80-1.21)</td>
<td>0.854</td>
</tr>
<tr>
<td>rs7240064</td>
<td>T(C)</td>
<td>-</td>
<td>-</td>
<td>0.27 (0.25)</td>
<td>0.268</td>
<td>1.01 (0.88-1.16)</td>
<td>0.875</td>
</tr>
</tbody>
</table>

All analyses are adjusted for baseline age, sex, ethnicity and treatment assignment. Allele effects are estimated within the pooled sample of three treatment groups, unless the test of treatment*SNP interaction was statistically significant (P<0.05), in which case the cells in the table are left blank and the relevant results are reported in Table 4.
Table 4

Treatment-specific SNP effects on short-term weight loss and diabetes risk.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor (major) allele</th>
<th>Lifestyle</th>
<th>Metformin</th>
<th>Placebo</th>
<th>6m Weight Loss (kg/allele)</th>
<th>2yr Weight Loss (kg/allele)</th>
<th>Diabetes Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Coefficient(SE)</td>
<td>P-value</td>
<td>Coefficient(SE)</td>
<td>P-value</td>
<td>Coefficient(SE)</td>
</tr>
<tr>
<td>rs17066856</td>
<td>C(T)</td>
<td>1041</td>
<td>-0.01 (0.33)</td>
<td>0.980</td>
<td>0.50 (0.27)</td>
<td>0.064</td>
<td>-0.33 (0.27)</td>
</tr>
<tr>
<td>rs9966412</td>
<td>A(G)</td>
<td>1024</td>
<td>0.15 (0.32)</td>
<td>0.640</td>
<td>0.54 (0.26)</td>
<td>0.034</td>
<td>-0.28 (0.25)</td>
</tr>
<tr>
<td>rs17066859</td>
<td>A(G)</td>
<td>1020</td>
<td>0.35 (0.39)</td>
<td>0.358</td>
<td>0.71 (0.30)</td>
<td>0.016</td>
<td>-0.36 (0.31)</td>
</tr>
<tr>
<td>rs8091237</td>
<td>G(C)</td>
<td>1012</td>
<td>-0.36 (0.32)</td>
<td>0.259</td>
<td>0.40 (0.24)</td>
<td>0.094</td>
<td>0.32 (0.26)</td>
</tr>
<tr>
<td>rs17066866</td>
<td>T(A)</td>
<td>1012</td>
<td>2.25 (0.77)</td>
<td>0.004</td>
<td>0.10 (0.60)</td>
<td>0.866</td>
<td>0.02 (0.60)</td>
</tr>
<tr>
<td>rs7240064</td>
<td>T(C)</td>
<td>1012</td>
<td>-0.11 (0.34)</td>
<td>0.758</td>
<td>0.29 (0.26)</td>
<td>0.266</td>
<td>0.64 (0.28)</td>
</tr>
<tr>
<td>rs17066829</td>
<td>A(T)</td>
<td>1082</td>
<td>0.80 (0.64-1.00)</td>
<td>0.054</td>
<td>1.06 (0.89-1.27)</td>
<td>0.514</td>
<td>1.09 (0.92-1.29)</td>
</tr>
</tbody>
</table>

All analyses are adjusted for baseline age, sex, ethnicity and treatment assignment.