TFAP2B Influences the Effect of Dietary Fat on Weight Loss under Energy Restriction

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Abstract

**Background:** Numerous gene loci are related to single measures of body weight and shape. We investigated if 55 SNPs previously associated with BMI or waist measures, modify the effects of fat intake on weight loss and waist reduction under energy restriction.

**Methods and Findings:** Randomized controlled trial of 771 obese adults. (Registration: ISRCTN25867281.) One SNP was selected for replication in another weight loss intervention study of 934 obese adults. The original trial was a 10-week 600 kcal/d energy-deficient diet with energy percentage from fat (fat%) in range of 20–25 or 40–45. The replication study used an 8-weeks diet of 880 kcal/d and 20 fat%; change in fat% intake was used for estimation of interaction effects. The main outcomes were intervention weight loss and waist reduction. In the trial, mean change in fat% intake was –12/+4 in the low/high-fat groups. In the replication study, it was –23/+12 among those reducing fat% more/less than the median. TFAP2B-rs987237 genotype AA was associated with 1.0 kg (95% CI, 0.4; 1.6) greater weight loss on the low-fat, and GG genotype with 2.6 kg (1.1; 4.1) greater weight loss on the high-fat (interaction p-value; p = 0.00007). The replication study showed a similar (non-significant) interaction pattern. Waist reduction results generally were similar. Study-strengths include (i) the discovery study randomised trial design combined with the replication opportunity (ii) the strict dietary intake control in both studies (iii) the large sample sizes of both studies. Limitations are (i) the low minor allele frequency of the TFAP2B polymorphism, making it hard to investigate non-additive genetic effects (ii) the different interventions preventing identical replication-discovery study designs (iii) some missing data for non-completers and dietary intake. No adverse effects/outcomes or side-effects were observed.

**Conclusions:** Under energy restriction, TFAP2B may modify the effect of dietary fat intake on weight loss and waist reduction.

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**Competing Interests:** Wim Saris is a part-time employee at DSM Nutritional Products Ltd, DSM Corporate Scientist; Francis Rousseau is a full time employee at IntegraGen SA; Arne Astrup is in the Medical Advisory Board of Pathway Genomics (La Jolla, California, USA); Stephan Rössner has received travel support, for some lectures on Qnexa, from VIVUS, Inc. (California, USA). A patent application has been filed by DSM, in the name of Thorkild IA Sørensen on behalf of the NUGENOB consortium, based on the findings described in this paper (status: a true patent application has been filed on 2011-11-28 and is scheduled to be published by the end of May, 2013). DSM (also referred to as the Innovation Centre in Urmond) has paid for the NUGENOB GWAS genotyping, which was
Introduction

Large genome-wide association studies (GWAS) have identified 32 gene loci in which single nucleotide polymorphisms (SNPs) are robustly, though moderately, related to body mass index (BMI) [1], and fewer variants have been related to proxy measures of abdominal obesity [2–4]. All these genetic variants have been related to body size measured at a single time point in adulthood. Individuals differ greatly in weight loss response to energy restriction [5–10]. A large part of the variation is likely explained by variation in adherence to a prescribed diet [9], but differences in physical activity and metabolic response determining energy efficiency may also play a role. Twin studies indicate that genetic predisposition influence weight change response during under- and overfeeding [11–13], but few studies have shown effects of various genotypes on weight loss response [14–20].

The optimal macronutrient composition of a diet for weight loss has been much debated [21,22]. We hypothesize that the individual genetic background may influence which diet is the most effective for weight loss. We previously investigated gene-macronutrient interactions in the NUGENOB (Nutrient-Gene Interactions in Human Obesity) study, in which obese men and women were randomized to a 10-weeks hypercocaloric diet with low or high fat relative to carbohydrate content. The assigned diets showed similar effects on weight loss [10], but we found that a SNP (rs7903146) of the TCF7L2 gene associated with diabetes modified the effect of randomized diet on weight loss [16]. However, we found no effect of the obesity-associated SNP of the FTO gene on weight loss [15], or, for the time, SNPs in a range of relevant candidate genes in obesity [23] on the diet-weight loss association.

The aim of this study was to examine the effect of SNPs in gene loci with an established association with body size or waist measures, on changes in weight and waist circumference. In NUGENOB we investigated main genetic effects and interactions between SNPs in these gene loci and the randomized low-versus high-fat group, and also for reported changes in energy intake from fat and in total, in relation to weight loss and waist reduction. In order to strengthen or refute findings of strong effects in NUGENOB, we aimed at replication in another weight loss study (Diet, Obesity and Genes, DiOGenes [24]).

Gene loci selection and genotyping

Gene loci included were those that had shown a consistent association with BMI, waist circumference, or waist-hip ratio in GWAS [1–4,25–35]. In total 55 SNPs were included in the study. The FTO SNP was genotyped by Taqman allelic discrimination (KBioScience, Herts, UK), with a 96.9% genotyping success rate and a genotyping error rate of 0.27%. Genome-wide genotyping on the Illumina 317 k quad chip carried out at IntegraGen, Evry, France, covered another 23 SNPs in this study, with a 99.1% genotyping success rate. The remaining 31 SNPs were genotyped by KASPar SNP Genotyping (KBioscience, Hoddesdon, UK). Success rate was above 97% and error rate was below 0.5% in 183 replicate samples. We used Fisher’s exact test to evaluate Hardy-Weinberg equilibrium (HWE) for all SNPs.

Statistical analysis

Drop-out rates by genotypes were compared by Pearson chi-square statistics. The baseline BMI of completers and non-completers were compared by two-sample t-test. We used linear regression to calculate main genotype effects, and gene-diet interactions, in relation to changes in weight and waist. The additive genetic effects and the sum of genotypes, each encoded 0, 1, or 2, equivalent to the number of obesity risk-alleles, were analyzed. Dietary factors investigated were the two randomized groups, and reported change in fat% and energy intake.

Individual change in dietary intake was calculated as the difference between mean intake over three days at baseline, and the intake over five days during the intervention, with equal weight for intake at the first part versus end of intervention. Change in weight and waist circumference corresponded to the difference between levels at baseline and directly after the intervention. All regression models included change in weight or waist as dependent variable, and explanatory variables were genotype, baseline weight or waist, sex, a product term for sex and baseline weight or waist, age, and study center. Analyses of gene-diet interactions additionally included a product term of genotype and the dietary factor of interest and also the dietary factor as main effect. Genetic effects
on weight change and waist change are denoted as effects on weight loss and waist reduction.

All statistical tests were two-sided and were performed in Stata 9.2 (StataCorp, LP, College Station, 2007).

Replication in DiOGenes

The strongest effects observed in NUGENOB were replicated in the DiOGenes study (www.diogenes-eu.org). The trial has been described in detail [24]; briefly, DiOGenes is an intervention study of obese adults undergoing two phases; an 8-weeks weight loss phase on a low-calorie diet, and 6–12 months weight maintenance on one of five diets. Data from the weight loss phase were used in the present study.

Participants were men and women with a BMI between 27 and 45 kg/m², recruited to one of eight European centers. Exclusion criteria were characteristics that could potentially influence the results, such as more than 3 kg weight change within two months prior to the study and medications or certain diseases. Participants of the study recorded their baseline dietary intake during two weekdays and one weekend day. Weighed food recordings were applied with instructions and procedures similar to those in NUGENOB. The 8-weeks weight loss intervention consisted of a low-calorie Modifast diet (Nutrition et Santé). Four items per day were selected from various products, each containing 202–218 kcal. Participants were also allowed a daily intake of 200 g tomatoes, 125 g cucumber, and 50 g lettuce. The low-calorie diet provided approximately 880 kcal/d with an energy-percent from fat of 20%, 54% from carbohydrates, and 26% from protein.

Anthropometric measurements were standardized across the study centers. Height was measured four weeks before the trial. Weight and waist circumference were measured 1–3 weeks before the trial and right after the intervention.

In total 934 participants started the weight loss trial in DiOGenes, and 790 of those had their DNA genome-wide genotyped; missing individuals were due to little or low-quality DNA. Genotyping was performed on the Illumina 660 k quad chip carried out at the Centre National de Génotypage (CNG), Evry, France. Genotyping was successful in 785 (99.4%) individuals. Quality control (duplicates, sex discrepancy, non-European, etc.) reduced the number to 734 (93%) and TFAP2B rs987237 was called for all.

Statistical analysis was conducted as described for NUGENOB. Dietary fat in gene-diet interaction analyses was in DiOGenes assessed for change in fat% intake, calculated as the difference between reported baseline intake and the common (approximate) fat proportion of the intervention diet. The inter-individual variation for calculated fat% change was therefore exclusively determined by baseline dietary intake. Median fat% change was used as cut-point for two groups of fat% change, which replaced the randomized fat groups in NUGENOB. Statistical models were identical in all other aspects.

Ethical considerations

The NUGENOB and DiOGenes trials have obtained written informed consent from all study participants, and ethical approval from the ethical committee at each study center. The names of the local ethics committees were: NuGenOb trial Research Ethics Committee of the University of Navarra, Spain; The Danish Research Ethics Committee System, Denmark; Medical Ethics Committee of the Maastricht University, Netherlands; Ethics Committee of the Hôtel-Dieu Hospital, France; University of Nottingham Medical School Ethics Committee, United Kingdom; The Swedish National Council on Medical Ethics, Sweden; Ethics Commission of the Medical School of Toulouse, France. (DiOGenes trial) Research Ethics Committee of the University of Navarra, Spain; The Danish Research Ethics Committee System, Denmark; Medical Ethics Committee of the Maastricht University, Netherlands; Ethics Committee of the University of Potsdam, Germany; Bedfordshire Local Research Ethics Committee, United Kingdom;

Research Ethics Committee of the University of Crete, Greece; Ethical Committee to the National Transport Multiprofile Hospital in Sofia, Bulgaria; Ethical Committee of the Faculty Hospital, Institute of Endocrinology, Prague, Czech Republic.

Results

NUGENOB

Out of 771 participants at start of the intervention, 648 (84%) completed it (Figure 1). Completers and non-completers did not differ significantly in baseline BMI ($P = 0.8$). One weight gainer with an extreme baseline BMI of 66 kg/m² and five participants with missing genotype data were excluded. For each of the 55 variants included in the study, genotype data were available in 617–642 participants (95–99% of completers). Genotype distributions were not related to drop-out rates except for ETI2 and FAM12, which had more completers among homozygotes than among non-carriers of the obesity risk-allele ($P = 0.01$ and 0.02 respectively), and for PTBP2, which showed the opposite pattern ($P = 0.03$). Of completers with genotype data, 580 (90% of 642) participants had dietary intake data available, and they were included in gene-diet interaction analyses that included reported dietary intake.

Participants had a mean baseline BMI of 35.5 kg/m², and mean weight was 96.8 kg in women and 110.6 kg in men (Table 1). Energy intake reduced by on average 615 kcal/d in the study group. This was achieved by a reduction in absolute intake of macronutrients, with a greater decrease in fat intake in the low-fat group, and a greater decrease of carbohydrates in the high-fat group. On average, women lost 6.5 kg weight and 5.9 cm waist and men lost 7.9 kg weight and 7.7 cm waist during the intervention.

The distribution of genotypes, allele frequency, and outcome of HWE test for the 55 gene loci are reported in Table S1. All minor allele frequencies were larger than 0.05, and HWE tests were acceptable with only one SNP (PFKP rs6602024) showing a $P$-value below 0.01.

Table 2 shows $P$-values for main effects and interactions with diet for 17 gene loci that reached nominal $P$-values below 0.05 for an association with weight loss or waist reduction. The most striking results were found for TFAP2B rs987237. This SNP modified the effect on weight loss by fat group with a nominal $P$-value for interaction of 0.0007, which also reached significance after correction for multiple testing of 55 SNPs (0.05/55 = 0.0009). In an additive gene-diet interaction model, homozygotes for the A allele lost 1.0 kg (95% confidence interval, CI, 0.4; 1.6) more weight on the low-fat than on the high-fat diet, whereas homozygotes for the G allele, i.e. the obesity risk-allele, lost 2.6 kg (95% CI, 1.1; 4.1) more weight on the high-fat diet (Figure 2). A similar but weaker pattern was observed for waist reduction ($P = 0.03$; Figure 3). TFAP2B was also directly related to weight loss ($P = 0.04$) with a 0.5 kg (95% CI, 0.0; 0.9) greater weight loss per G allele.

Three other gene loci showed $P$-values between 0.0003 and <0.01 for a main effect or interaction with diet, in relation to weight loss or waist reduction (Table S2). These were main effects on weight loss by VEGFA, and a gene-fat group interaction in relation to waist reduction for CTNNBL1 and NPC1.

The gene score (sum of all the risk alleles over genotypes) showed no main effect or interaction with diet in relation to outcomes ($P = 0.09$).
Table 1. Descriptive information with respect to the NUGENOB study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 481)</th>
<th>Men (n = 161)</th>
<th>All (n = 642)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change*</td>
<td>Baseline</td>
</tr>
<tr>
<td>Age, years</td>
<td>36.5 (7.9)</td>
<td>38.7 (7.6)</td>
<td>37.1 (7.9)</td>
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<tr>
<td>Weight, kg</td>
<td>96.8 (14.5)</td>
<td>-6.5 (3.2)</td>
<td>110.6 (16.3)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>35.8 (4.8)</td>
<td>-2.4 (1.2)</td>
<td>34.7 (4.5)</td>
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<tr>
<td>Waist circumference, cm</td>
<td>103.2 (12.0)</td>
<td>-5.9 (4.5)</td>
<td>113.9 (11.4)</td>
</tr>
<tr>
<td>Low-fat diet (n = 298)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake, kcal/d</td>
<td>2201 (691)</td>
<td>-652 (632)</td>
<td>2194 (625)</td>
</tr>
<tr>
<td>Fat, E%</td>
<td>36.7 (7.6)</td>
<td>-11.6 (8.2)</td>
<td>37.0 (7.8)</td>
</tr>
<tr>
<td>Fat, g</td>
<td>90.9 (37.3)</td>
<td>-47.4 (34.8)</td>
<td>91.2 (35.7)</td>
</tr>
<tr>
<td>Carbohydrates, E%</td>
<td>45.2 (8.6)</td>
<td>11.5 (8.9)</td>
<td>44.8 (8.7)</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>246.7 (86.4)</td>
<td>-27.2 (85.3)</td>
<td>245.0 (80.2)</td>
</tr>
<tr>
<td>Protein, E%</td>
<td>16.3 (3.4)</td>
<td>1.6 (3.6)</td>
<td>16.3 (3.2)</td>
</tr>
<tr>
<td>Protein, g</td>
<td>87.7 (27.6)</td>
<td>-18.7 (26.0)</td>
<td>88.4 (27.6)</td>
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</table>

Mean (SD) baseline level and change of body size by sex in 642 completers in NUGENOB with genotype data available, and dietary intake by randomized group in 580 completers with genotype data and dietary intake data available.

E%: percent of total energy intake.

*Difference between baseline and after the intervention.

†Data were missing in 14 participants.

‡Difference between reported intake at baseline and during the intervention.

Alcohol contributed with on average 1.8 E% (6.3 g) at baseline and the proportion reduced during the intervention.
Replication of TFAP2B-fat interaction analyses in DiOGenes

Out of 803 (86%) completers of the intervention, data on TFAP2B were available in 639 participants, of which 590 participants had reported their dietary intake before the intervention. Completers did not differ significantly from non-completers with respect to BMI (P = 0.7) or TFAP2B genotype distribution (P = 0.4). Characteristics of DiOGenes participants are shown in Table 3. Whereas the range for change in fat% intake in NUGENOB was broad and largely related to randomized group, fat intake among DiOGenes participants reduced much both in terms of absolute intake (mean: -274.1 g/d, range: -251.2 to -0.1 g) and fat%
Figure 2. Weight loss over *TFAP2B* rs987237 genotypes in NUGENOB. Effect of randomized fat group (low-fat, LF, and high-fat, HF) on weight loss in NUGENOB by *TFAP2B* rs987237. The y-axis displays the mean weight loss in each group. *P*-values for interaction, and effect estimates, were derived from linear regression, based on the assumption of an additive genetic model.

doi:10.1371/journal.pone.0043212.g002

Figure 3. Waist reduction over *TFAP2B* rs987237 genotypes in NUGENOB. Effect of randomized fat group (low-fat, LF, and high-fat, HF) on waist reduction in NUGENOB by *TFAP2B* rs987237. The y-axis displays the mean waist reduction in each group. *P*-values for interaction, and effect estimates, were derived from linear regression, based on the assumption of an additive genetic model.

doi:10.1371/journal.pone.0043212.g003
Table 3. Descriptive information with respect to the DiOGenes study.

<table>
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<tr>
<th>Variable</th>
<th>Women (n=415)</th>
<th>Men (n=224)</th>
<th>All (n=639)</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Change*</td>
<td>Baseline</td>
</tr>
<tr>
<td>Age, years</td>
<td>41.0 (6.3)</td>
<td>43.0 (5.5)</td>
<td>42.0 (6.1)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>94.9 (15.4)</td>
<td>-9.9 (3.0)</td>
<td>109.5 (17.3)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>34.5 (4.9)</td>
<td>3.7 (1.0)</td>
<td>34.4 (4.6)</td>
</tr>
<tr>
<td>Waist circumference, cm†</td>
<td>104.3 (11.8)</td>
<td>-9.1 (4.7)</td>
<td>114.6 (12.4)</td>
</tr>
<tr>
<td>&gt;16.7 fat% decrease(n=295)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake, kcal/d</td>
<td>2307 (774)</td>
<td>-1427 (774)</td>
<td>2154 (778)</td>
</tr>
<tr>
<td>Fat, E%†</td>
<td>43.5 (5.1)</td>
<td>-23.0 (5.1)</td>
<td>32.0 (3.8)</td>
</tr>
<tr>
<td>Carbohydrates, E%‡</td>
<td>38.0 (6.2)</td>
<td>15.7 (6.2)</td>
<td>47.6 (7.2)</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>218.1 (77.6)</td>
<td>-100.1 (77.6)</td>
<td>257.2(100.3)</td>
</tr>
<tr>
<td>Protein, E%‡</td>
<td>17.1 (4.0)</td>
<td>8.8 (4.0)</td>
<td>17.0 (4.5)</td>
</tr>
<tr>
<td>Protein, g</td>
<td>96.5 (34.5)</td>
<td>-39.5 (34.5)</td>
<td>88.3 (30.3)</td>
</tr>
</tbody>
</table>

Mean (SD) baseline level and change of body size by sex in 639 completers in DiOGenes in whom TFAP2B was successfully genotyped, and dietary intake in groups split by median change in fat% intake in 590 completers with TFAP2B data and dietary intake data available. E% percent of total energy intake.

*Difference between baseline and after the intervention.
  1Data were missing in 41 participants.
  2One participant reported an increased (1.7 units) fat% intake.
  3Difference between reported intake at baseline and the standardised low-calorie diet of 880 kcal/day, including macronutrients: fat, 20 g, 20 E%; carbohydrates, 118 g, 54 E%; and protein, 57 g, 26 E%.

Table 3 indicates that under energy restriction, TFAP2B may influence the effect of dietary fat on weight loss.

Several factors may underlie the much weaker associations observed in the replicated analysis. Analyses in NUGENOB were performed in line with the original design; two randomized groups of low or high fat% intake were compared. Besides the control of known confounders owing to randomization, the two groups in NUGENOB were also more diverse in their changed fat% intake than were the constructed groups in DiOGenes with different decreases in dietary fat% intake. Moreover, we lacked information on the actual individual dietary intake during the intervention in DiOGenes, so dietary change calculations assumed complete adherence to the prescribed diet and this assumption is likely not to be entirely met. Given these limitations to enable replication of the findings in NUGENOB, the similar pattern observed in DiOGenes is quite noteworthy. Many of the same limitations in DiOGenes confer also to NUGENOB for investigation of change in fat% and energy intake. Misclassification of self-reported dietary intake, small variation in exposure or small differences between compared groups, and confounding issues may have obscured the finding of further strong gene-diet interactions in NUGENOB, and a clearer replication of TFAP2B-dietary fat interaction in DiOGenes.

Other limitations of the study are the incomplete data due to drop-outs of study participants, and incomplete genotyping and dietary intake reports. Around 15% of study participants did not complete the trials; however, completers and non-completers did neither differ in baseline BMI nor in overall genotype distribution. Dietary intake data were incomplete in both studies, but were based on weighed food records over several days that, despite its
Figure 4. Weight loss over TFAP2B rs987237 genotypes in DiOGenes. Effect of fat% reduction on weight loss in DiOGenes by TFAP2B rs987237. The two groups of fat% reduction were divided by the median reduction of 16.7 energy percent. The y-axis displays the mean weight loss in each group. P-values for interaction, and effect estimates, were derived from linear regression, based on the assumption of an additive genetic model. doi:10.1371/journal.pone.0043212.g004

Figure 5. Waist reduction over TFAP2B rs987237 genotypes in DiOGenes. Effect of fat% reduction on waist reduction in DiOGenes by TFAP2B rs987237. The two groups of fat% reduction were divided by the median reduction of 16.7 energy percent. The y-axis displays the mean waist reduction in each group. P-values for interaction, and effect estimates, were derived from linear regression, based on the assumption of an additive genetic model. doi:10.1371/journal.pone.0043212.g005
weaknesses owing to self-report, is frequently used as gold standard method for assessment of dietary intake [36].

The major strength of the study is the relatively large sample size of two well-controlled intervention studies of similar participant characteristics, allowing replication of analyses for highly significant findings. Replication is crucial to avoid spurious chance findings emerging from multiple testing. Although analyses in DiOGenes did not replicate the strong findings in NUGENOB, the patterns of results were consistent.

The TFAP2B gene has been associated with type 2 diabetes [37], and was recently found to be related to BMI [1] and waist circumference [4]. The TFAP2B gene encodes for a transcriptional factor-activating enhancer-binding protein-2 (AP-2β) and is preferentially expressed in adipose tissue [37]. Overexpression of TFAP2B leads to increased glucose uptake and thereby triglyceride accumulation and insulin resistance in adipocytes [38]. The rs987237 variant investigated in this study, located in intron 3, may not be the causal variant. It may, however, be a marker of the enhancer activity, since it is in complete linkage disequilibrium (r² = 1) with an intronic enhancer variant that influences TFAP2B expression in adipose tissue [39]. Yet, the mechanisms whereby TFAP2B could modify the effect of dietary fat intake on weight loss are unclear. It is also unclear whether TFAP2B indeed interacts with dietary fat, as focused on in our paper, or rather with carbohydrates, or the fat-carbohydrate ratio. Speculatively, individuals of different TFAP2B genotype and gene expression metabolise fat or other macronutrients differently, and thus, respond differently to dietary change of macronutrient composition.

In conclusion, this study of obese men and women showed a clear interaction between TFAP2B and a diet low or high in fat, on weight loss under energy restriction. A similar, but nonsignificant, pattern for interaction between TFAP2B and dietary fat intake was shown in another weight loss study of similar participant characteristics. Results of our study strongly encourage further examination of the role of TFAP2B and macronutrients in weight loss.

**Supporting Information**

**Figure S1** Weight loss over TFAP2B rs987237 genotypes in NUGENOB based on general genetic model. Effect of randomized fat group (low-fat, LF, and high-fat, HF) on weight loss in NUGENOB by TFAP2B rs987237. The y-axis displays the mean weight loss in each group. P-values for interaction, and effect estimates, were derived from linear regression and can be found in Table S3 and S4. Gene with interesting findings (SNP coded as a categorical variable). (PDF)

**Figure S2** Waist reduction over TFAP2B rs987237 genotypes in NUGENOB based on general genetic model. Effect of randomized fat group (low-fat, LF, and high-fat, HF) on waist reduction in NUGENOB by TFAP2B rs987237. The y-axis displays the mean waist reduction in each group. P-values for interaction, and effect estimates, were derived from linear regression and can be found in Table S3 and S4. Gene with interesting findings (SNP coded as a categorical variable). (PDF)

**References**


