

Genetic Polymorphisms and Weight Loss in Obesity: A Randomised Trial of Hypo-Energetic High- versus Low-Fat Diets

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Abbreviations: CI, confidence intervals; NUGENOB, Nutrient-Gene Interactions in Human Obesity: Implications for Dietary Guidelines; WL, weight loss

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ABSTRACT

Objectives: To study if genes with common single nucleotide polymorphisms (SNPs) associated with obesity-related phenotypes influence weight loss (WL) in obese individuals treated by a hypo-energetic low-fat or high-fat diet.

Design: Randomised, parallel, two-arm, open-label multi-centre trial.

Setting: Eight clinical centres in seven European countries.

Participants: 771 obese adult individuals.

Interventions: 10-wk dietary intervention to hypo-energetic (−600 kcal/d) diets with a targeted fat energy of 20%–25% or 40%–45%, completed in 648 participants.

Outcome Measures: WL during the 10 wk in relation to genotypes of 42 SNPs in 26 candidate genes, probably associated with hypothalamic regulation of appetite, efficiency of energy expenditure, regulation of adipocyte differentiation and function, lipid and glucose metabolism, or production of adipocytokines, determined in 642 participants.

Results: Compared with the noncarriers of each of the SNPs, and after adjusting for gender, age, baseline weight and centre, heterozygotes showed WL differences that ranged from −0.6 to 0.8 kg, and homozygotes, from −0.7 to 3.1 kg. Genotype-dependent additional WL on low-fat diet ranged from 1.9 to −1.6 kg in heterozygotes, and from 3.8 kg to −2.1 kg in homozygotes relative to the noncarriers. Considering the multiple testing conducted, none of the associations was statistically significant.

Conclusions: Polymorphisms in a panel of obesity-related candidate genes play a minor role, if any, in modulating weight changes induced by a moderate hypo-energetic low-fat or high-fat diet.

Editorial Commentary

Background: Obesity is an important cause of death and disease, particularly in the developed world. It is understood that both environmental and genetic factors contribute towards obesity. Numerous studies have associated particular gene variants with a tendency towards obesity, but it is not known whether such gene variants affect the degree to which obese individuals will lose weight when dieting.

What this trial shows: As part of a randomised trial, 771 participants were assigned to one of two different low-energy diets for 10 weeks: one low in fat or one high in fat. The researchers then did a genetic analysis of 642 participants completing the intervention, to find out whether any of 42 distinct genetic variations in 26 genes were associated with weight loss in the trial. The genetic variants were chosen for study as they were known or already thought to be associated with appetite regulation or various aspects of metabolism and fat tissue development and function. The investigators found that none of the genetic variants studied had a significant association with weight loss in the trial. It was also seen that the majority of genetic variants were not associated with efficacy of one dietary intervention over another.

Strengths and limitations: Although a large number of participants was recruited into the trial, the genetic analysis involved multiple comparisons—168 tests of 42 genetic variants. This increases the likelihood that any significant associations found could have resulted from chance alone. Significant associations from this study will require additional confirmation in larger studies.

Contribution to the evidence: This study adds data indicating that variation in the genes studied did not have an important influence on weight loss.

The Editorial Commentary is written by PLoS staff, based on the reports of the academic editors and peer reviewers.

INTRODUCTION

Obesity has a joint genetic and environmental etiology [1]. Rare cases of severe, early onset obesity have been attributed to single gene mutations with large functional effect [2]. The genetic influence on obesity may be because of combinations of multiple genes with individual small effect sizes related to frequent single nucleotide polymorphisms (SNPs). These may interact mutually and with the environment in a complex manner to influence obesity [2,3]. Numerous gene variants have been reported to be associated with obesity or obesity-related phenotypes [4], but only a few of the associations have been replicated in other populations [2,4].

It is conceivable that the effect of some genes on the obesity phenotypes may be nutrient-sensitive. Indeed, diet composition and/or energy intake may modulate gene expression through complex transcriptional mechanisms as well as more downstream processes involving the gene products [2,3]. Several studies have clearly demonstrated that expression of multiple genes in adipose tissue is altered by reductions in energy intake, but so far none has revealed an influence of the dietary composition in this setting [3,5,6]. The implications for human health of alterations in these processes by common genetic polymorphisms are still elusive, but they may be revealed by showing that the effect of the diet on weight changes depends on differences in the genes, and this could also help explain discrepancies in the association studies.

However, few gene-nutrient interaction studies of obesity and weight change have been carried out in humans, and the

results have been ambiguous, whether being based on either epidemiological, observational, and cross-sectional studies [3,7] or small experimental over-feeding studies [8]. A few studies [3], including twin studies [9], suggest that genes play a role in weight loss (WL) as the outcome of dietary treatment of obesity. These genes could be the same genes as those involved in obesity, exemplified by a study of the relation between variation in the *MC4R* gene, the most common form of monogenic obesity [2,10], and the outcome of surgical treatment [11]. However, twin studies of the two phenotypes—degree of obesity and WL—show that the genetic correlation for these phenotypes is far from 1.0, implying that it is only a subset of the genes that are the same behind the genetic influence on either of the two phenotypes [12].

A possibly useful approach to elucidate the role of the genes and diets in obesity would be to study effects of genetic polymorphisms on WL induced by specific dietary treatments of obesity. The NUGENOB Consortium (Nutrient-Gene Interactions in Human Obesity: Implications for Dietary Guidelines) has conducted a randomised intervention trial of a 10-wk low-fat or high-fat hypo-energetic diet (with fixed protein content and a corresponding high or low carbohydrate content) in obese individuals from eight centres in seven European countries with the specific purpose of studying aspects of gene-nutrient interaction (for more on the NUGENOB project, see <http://www.nugenob.org>) [13]. This trial showed that the low-fat diet produced the same mean WL as the high-fat diet, but resulted in more participants losing >10% of initial body weight and had fewer dropouts [13].

The aim of the present study was to investigate, using the NUGENOB trial [13], if genetic polymorphism modulates WL induced by either a low- or a high-fat hypo-energetic diet. Common SNPs in a panel of obesity-related candidate genes were selected on the basis of their known or presumed involvement in various parts of the pathogenic processes of obesity and its related phenotypes.

METHODS

The study was a randomised, parallel, two-arm, open-label, 10-wk intervention by two hypo-energetic diets with either low or high fat content, undertaken at eight sites in seven European countries. The clinical parts of the trial are described in detail in a previous publication [13].

Participants and Baseline Investigation

We planned to recruit 100 Caucasian Europeans from each of seven centres and 50 from one other centre, and included during the period May 2001 through September 2002 in total 771 participants (579 women) from the United Kingdom (Nottingham), the Netherlands (Maastricht), France (Paris and Toulouse), Spain (Pamplona), Czech Republic (Prague), Sweden (Stockholm), and Denmark (Copenhagen).

The study aimed at recruiting participants with a body mass index (BMI) greater than or equal to 30 kg/m² and age 20–50 y, and without weight change more than 3 kg within the 3 mo prior to the study start. Participants reporting clinically diagnosed hypertension, diabetes or hyperlipidaemia treated by drugs, untreated thyroid disease, surgically or drug-treated obesity, pregnancy, or alcohol or drug abuse were excluded. Participants not included in other trials were recruited

through local sources as available and convenient. The ethical committee at each of the participating centres approved the study. Volunteers were informed about the nature of the study, and written consent was obtained prior to study participation.

Before assignment to the dietary intervention group, all participants underwent a thorough standardised clinical and physiological examination described in Standard Operational Procedures applied in all participating centres (see Trial Protocol and Annexes). Each examination included blood sampling, and measurement of height with a calibrated stadiometer and weight (in light indoor clothes and without shoes) with a calibrated set of scales. Resting metabolic rate was measured by a ventilated hood system.

Blood drawing and processing of blood samples were performed according to international guidelines for genetic studies [14]. Whole blood was drawn in sterile vacutainer tubes with ethylenediaminetetraacetic acid. The plasma was removed, and the white cell layer (the buffy coat) was transferred to cryovials and frozen at -80°C .

Interventions

The target macronutrient composition of the two diets was: *low-fat diet*: 20%–25% of total energy from fat, 15% from protein and 60%–65% from carbohydrate; *high-fat diet*: 40%–45% of total energy from fat, 15% from protein, and 40%–45% from carbohydrate. Both diets were designed to provide 600 kcal/d fewer than the individually estimated energy requirement, which was based on pre-treatment resting metabolic rate multiplied by a physical activity level of 1.3, assuming a sedentary life style. The dietary programme is described in detail on the Web site <http://www.nugenob.org>. The dietary instructions also aimed at minimising differences between the two diets in other components such as source and type of fat, amount and type of fibre, type of

carbohydrate, amount of fruit and vegetables, and in meal frequency patterns, while taking local customs into account as appropriate. Participants were requested to abstain from alcohol consumption. The dietary instructions were reinforced and monitored and participants were weighed weekly. Participants were advised to follow their habitual activity patterns throughout the dietary intervention period. A 3-d, weighed food record of two weekdays and one weekend day was obtained before the study and during the last week of the intervention. One-day weighed food records were completed during the second, fifth, and seventh weeks. The dietary records were analysed using the country-specific food-nutrient database routinely used in each centre. The nutrient composition of the baseline diet was the average across the 3d of recordings, and the composition of the intervention diet was based on averaging across all the dietary recordings during the intervention period (Table 1).

Objectives

The objectives of the clinical trial were to assess the effect on WL in obese participant of a low-fat versus a high-fat hypo-energetic diet. The objectives of the present analysis were to investigate if the genotypes of 42 SNPs in 26 candidate genes, probably associated with hypothalamic regulation of appetite, efficiency of energy expenditure, regulation of adipocyte differentiation and function, lipid and glucose metabolism, or production of adipocytokines, influenced the WL, possibly dependent on which of the two diets the participants were prescribed.

Outcomes

The outcomes in the present analysis were the WL during the 10-wk dietary intervention in the various genotype groups defined by the SNPs in the selected genes.

The selection of candidate genes was based on their

Table 1. Baseline and 10-wk Measurements of Body Weight, Body Mass Index, Energy Intake, and Macronutrient Energy Percent of the Diet (without Alcohol Energy Percent) in 642 European Obese Individuals Who Completed Dietary Intervention

Measurement	Group	High-Fat Diet ^a		Low-Fat Diet ^b	
		Baseline	Decrease	Baseline	Decrease
Body weight (kg)	Women	97.0 (13.8)	6.2 (3.3)	96.6 (15.1)	6.7 (3.1)
	Men	111.0 (14.8)	8.2 (3.5)	110.3 (17.6)	7.7 (4.0)
	All	100.5 (15.3)	6.7 (3.5)	100.1 (16.9)	6.9 (3.4)
Body mass index (kg/m ²)	Women	35.7 (4.6)	2.3 (1.2)	35.8 (4.9)	2.5 (1.1)
	Men	34.9 (4.3)	2.6 (1.1)	34.5 (4.8)	2.4 (1.3)
	All	35.5 (4.6)	2.4 (1.2)	35.5 (4.9)	2.5 (1.2)
Energy intake (kcal/d)	Women	2,043 (568)	528 (536)	2,047 (550)	601 (531)
	Men	2,677 (724)	715 (637)	2,692 (917)	805 (895)
	All	2,199 (667)	574 (567)	2,209 (717)	653 (647)
Fat energy percent in diet	Women	36.5 (7.7)	−4.3 (8.5)	36.4 (7.0)	11.9 (7.8)
	Men	37.3 (8.1)	−3.9 (9.9)	37.0 (8.4)	11.0 (8.8)
	All	36.7 (7.8)	−4.2 (8.9)	36.6 (7.4)	11.7 (8.1)
Carbohydrate energy percent in diet	Women	45.7 (8.8)	3.6 (9.4)	45.7 (7.9)	−11.5 (8.7)
	Men	42.7 (8.5)	1.6 (9.8)	43.9 (9.9)	−12.0 (9.8)
	All	44.9 (8.6)	3.1 (9.5)	45.3 (8.5)	−11.6 (9.0)
Protein energy percent in diet	Women	16.5 (3.6)	−0.6 (3.9)	16.7 (3.8)	−1.4 (4.2)
	Men	16.9 (3.2)	−0.2 (3.0)	16.3 (3.2)	−1.4 (3.2)
	All	16.6 (3.5)	−0.5 (3.7)	16.6 (3.7)	−1.4 (4.0)

Baseline values and decreases are means (SD).

^a*n* = 232 women, 76 men.

^b*n* = 249 women, 85 men.

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potential contribution to obesity-related phenotypes, their presumed nutrient-sensitive expression and function of the gene products, and the presence of common SNPs with a presumed allele frequency above 0.05 allowing meaningful analysis of the interaction between diet and its effect on WL. The SNPs were selected through mutation analysis of genes found by positional cloning, mutation analysis of plausible biological candidate genes selected from the literature and found to be associated with functional effects of the gene products, and the available pertinent knowledge about already known obesity-related SNPs [2].

On this basis, 42 SNPs in 26 genes known or presumed to be associated with hypothalamic regulation of appetite, efficiency of energy expenditure, regulation of adipocyte differentiation and function, lipid and glucose metabolism, or production of several adipocytokines were selected [15–54]. Table 2 lists the genes and the SNPs analysed, and they are grouped according to their presumed function in relation to the obesity phenotypes. It appears from the positions of the SNPs that some may have functional effects by changing the gene products, some may affect the promoter function, and others may be in linkage disequilibrium with other SNPs with functional effects. Since all selected SNPs have been found in previous studies to be associated with obesity-related phenotypes, they may be considered tag SNPs of potentially functional haplotypes irrespective of their own presumed functional effects in relation to the obesity-related phenotypes or the currently addressed induced WL. For several of the genes selected (*ENPP1*, *GAD2*, *CART*, *SLC6A14*, *GHRL*, *PCSK1*, *ADIPOQ*, and *WAC*) a linkage disequilibrium analysis secured an optimal coverage of the genetic variation known at the time of gene selection. For this reason, these SNPs were considered the risk SNPs, and the participants not carrying these SNPs were denoted “noncarriers” corresponding to “wild types” irrespective of which alleles were most frequent in the present study.

For each genotype, four genetic models were analysed. In the first general genetic model, there were no assumptions made about a specific effect of the mutant alleles in the individuals who were heterozygous and homozygous for the gene variant when compared with those who were noncarrier (null hypothesis). Three specific genetic models assume a particular effect of the gene variant compared with the noncarrier: dominant effect (noncarrier = 0, heterozygous and homozygous = 1), co-dominant effect (assessed as an additive effect: noncarrier = 0, heterozygous = 1, homozygous = 2), and recessive effect of gene variant (noncarrier = 0, heterozygous = 0, homozygous = 1). For some of the variants, the homozygotes were so rare (less than 10 homozygotes in 7 out of the 42 SNPs) that identification of recessive transmission or distinction between dominant and co-dominant transmission would not be possible (Table 2).

Genotyping

Samples of buffy coat, drawn at baseline, were sent to the Steno Diabetes Centre in Copenhagen on dry ice, where DNA was extracted. Extracted DNA samples were diluted in Tris/EDTA buffer to a stock DNA solution of 100 ng/μl and a working DNA solution of 10 ng/μl. Stock solutions were stored at –80 °C, working solutions were stored at 4 °C. DNA samples were stored and handled in locations free of contaminating polymerase chain reaction products.

Forty of the 42 SNPs in the 26 genes were genotyped by the LightCycler assay (Roche Diagnostics, Basel, Switzerland) (the *SLC6A14*, *CART*, *GHSR*, *GAD2*, *GHRL*, *LEPROTL1*, *UCP2*, *UCP3*, *WAC*, *LIPC*, *IGF2*, *ENPP1*, *ADIPOQ*, and *CD36* genes) based on hybridization probes labelled with fluorescent dyes that allow fluorescence resonance energy transfer [55] or by TaqMan assay (Applied Biosystems, Foster City, California, United States) (the *MKKS*, *PCSK1*, *FOXC2*, *PPARGCIA*, *PPARG2*, *PPARG3*, *SREBF1*, *HSD11B1*, *KCNJ11*, *IL6*, *TNF α* , and *SERPINE1* genes). Sequences of primers pairs, labelled with fluorescein and LC Red 640 are available on request from the authors. One SNP in *IGF2* (–6815 A>T) and one in *CART* (–3608 T>C) were genotyped by direct sequencing. No genotyping could be carried out in seven out of the 771 participants enrolled in the study, and in five out of those completing the intervention. The genotyping success rate was 92.8%–98.8% except for *WAC* (89.2%) and *SERPINE1* (86.6%).

Sample Size

For a single analysis, the least detectable effect (WL, in kg) was estimated by the programme QUANTO version 1.0 (see <http://hydra.usc.edu/gxe>) with the assumptions of a statistical power of 0.80, a sample size of 642 individuals, and a mean WL of 6.8 kg with a SD of 3.5 kg. For allele frequencies ranging from 0.05–0.50, the least detectable WL of main effects of genes ranged from 1.30–0.78 kg (0.89 kg for an allele frequency of 0.50) for dominant models, from 7.72–0.89 kg for recessive models, and from 1.25–0.55 kg for additive co-dominant models. Assuming, in addition, equal distribution of participants between the diets, the least detectable effects on WL of gene-diet interaction, modelled as specified above, ranged from 2.59–1.54 kg (1.77 kg for allele frequency of 0.50) for dominant models, from 15.5–1.77 kg for recessive models, and from 2.49–1.07 kg for additive co-dominant models.

When assuming that testing for each SNP in each genetic model is a testing of the same null hypothesis multiple times, the needed sample size increases. Thus, as an example, detection of a significant difference of 1.5 kg between the low-fat and high-fat diets in heterozygotes and a difference of 3.0 kg between the diets in homozygotes versus that observed in noncarriers at an alpha level of 0.05, and a statistical power of 0.80 with four genetic models for 42 SNPs (168 tests of the same null hypothesis) would require sample sizes ranging from 5,729 through 1,008 participants in the trial for allele frequencies ranging from 0.05 through 0.50, respectively.

Randomisation

Stratified block randomisation was used with centre, gender, and three age groups (20–29 y, 30–39 y and 40–50 y) as strata and a block size of 12. The randomisation list was computer-generated and the block size was unknown to the clinical centres. Individual randomisation was carried out at the co-ordinating centre following transfer from the clinical centres of the relevant baseline information on the participants.

Statistical Methods

Examination of Hardy-Weinberg equilibrium was carried out by applying a global test for all genotypes (using the HWE.GENEPop programme) [56], and a test for each genotype by taking into account centre differences by summing up Pearson χ^2 statistics for each centre and

Table 2. Genotype Distribution and Allele Frequencies in 642 European Obese Women and Men Enrolled in the NUGENOB Study

Presumed Function of Genes	Normal Name	Gene Name	Locus ID	Location	Rs	SNP	Genotype		Hardy-Weinberg Equilibrium ^b		
							Non-carrier	Heterozygote	Homozygote	Allele Frequency	p-Value
Hypothalamic regulation of appetite	Solute carrier family 6 member 14 [1] Women ^a	SLC6A14	11254	Xq23	2011162	+22510 C>G	79	219	153	0.58	0.70
	Solute carrier family 6 member 14 [1] Men ^a	SLC6A14	11254	Xq23	2011162	+22510 C>G	66	—	80	0.55	—
Cocaine- and amphetamine-regulated transcript [2]	Cocaine- and amphetamine-regulated transcript [2]	CART	9607	5q13.2	7379701	-3608 T>C[2]	150	305	170	0.52	0.58
							221	292	122	0.42	0.19
							155	303	163	0.51	1.00
Growth hormone secretagogue receptor [3,4]	Growth hormone secretagogue receptor [3,4]	GHR	2693	3q26.3	2232169	Leu149Leu	512	105	6	0.094	0.53
							599	25	0	0.022	1.00
Glutamate decarboxylase 2[5]	Glutamate decarboxylase 2[5]	GAD2	2572	10p11.23	928197	+83897 T>A	426	169	31	0.18	0.0065
							318	257	57	0.29	0.12
							416	173	30	0.19	0.30
Ghrelin[6,7]	Ghrelin[6,7]	MKKS	8195	20p12	1547	Arg517Cys	552	69	4	0.062	0.56
							505	120	1	0.097	0.57
Leptin receptor overlapping transcript-like 1 [9]	Leptin receptor overlapping transcript-like 1 [9]	LEPROTL1	23484	8p21.2-1	—	-2625 C>T	551	54	1	0.046	0.48
							360	215	47	0.25	0.074
Efficiency of energy expenditure	Protein convertase subtilisin/kexin type 1[10]	PCSK1	5122	5q15	6235	T690S C>G	250	307	81	0.37	0.50
	Uncoupling protein 2[11]	UCP2	7351	11q13	6593669	-866 G>A	351	239	46	0.26	0.94
Regulation of adipocyte differentiation and function	Uncoupling protein 3[12]	UCP3	7352	11q13	1900849	-55 C>T	86	288	227	0.62	0.31
	Forkhead box C2[13,14]	FOXO2	2303	16q22-24	—	-512 C>T	74	276	276	0.66	0.83
Peroxisome proliferative activated receptor. Gamma coactivator 1. alpha[15,16]	Peroxisome proliferative activated receptor. Gamma coactivator 1. alpha[15,16]	PPARGC1A	10891	4p15.1	8192678	Gly482Ser[15]	178	298	151	0.48	0.73
							475	151	12	0.14	0.30
Peroxisome proliferative activated receptor. Gamma isoform 2	Peroxisome proliferative activated receptor. Gamma isoform 2	PPARG2	5468	3p25	1801282	Pro12Ala[17,18]	474	144	11	0.13	0.66
							465	147	13	0.14	0.64
Peroxisome proliferative activated receptor. Gamma isoform 3	Peroxisome proliferative activated receptor. Gamma isoform 3	PPARG3	5468	3p25	10865710	-681C>G[22]	346	258	37	0.26	0.57
							248	304	83	0.37	0.59
Sterol regulatory element binding transcription factor 1[23]	Sterol regulatory element binding transcription factor 1[23]	SREBF1	6720	17p11.2	—	17 C>G	142	274	157	0.51	0.16
							142	274	157	0.51	0.16
WW domain containing adaptor with coiled-coil[24]	WW domain containing adaptor with coiled-coil[24]	WAC	51322	10p12.1	2807761	-829 A>G	142	274	157	0.51	0.16
							142	274	157	0.51	0.16

Table 2. Continued

Presumed Function of Genes	Normal Name	Gene Name	Locus ID	Location	Rs	SNP	Genotype		Hardy-Weinberg Equilibrium ^b		
							Non-carrier	Hetero-zygote	Homo-zygote	Allele Frequency	p-Value
Regulation of lipid and glucose metabolism	Hydroxysteroid (11-beta) Dehydrogenase 1[25]	<i>HSD11B1</i>	3290	1q32.2	846919	-2940 G>A[26]	525	70	1	0.060	0.97
	Hepatic lipase C[27]	<i>LIPC</i>	3990	15q21-23	6082	Asn215Ser -514 C>T[27]	280	282	71	0.33	0.91
	Insulin-like growth factor 2	<i>IGF2</i>	3481	11p15.5	3168310	1926 C>G[28] Apol A>G[28,29]	308	256	59	0.30	0.88
Potassium inwardly rectifying channel J11 (KIRG.2)	Ectonucleotide pyrophosphatase/phosphodiesterase 1[32]	<i>KCNJ11</i>	3767	11p15.1	5219	Glu23Lys[30,31]	241	300	73	0.36	0.26
Production of adipocytokines	Adiponectin[33,34]	<i>ADIPOQ</i>	9370	3q27.3	1044498	Lys121Gln	433	171	12	0.16	0.84
					7754561	1044 A>G	318	244	40	0.27	0.34
					266729	-11377 C>G	356	236	38	0.25	0.32
					2241766	+45 T>G	477	130	21	0.14	0.052
					1501299	+276 T>G	76	233	323	0.70	0.021
CD36 antigen[35]	Interleukin 6[36,37]	<i>ADIPOQ</i>	948	7q11.2	17300539	-11391 G>A	525	101	3	0.085	0.78
					2232169	-178 A>C	110	294	189	0.57	0.68
					1800795	-174 G>C	184	326	116	0.45	0.62
					1800629	-308 G>A	459	159	14	0.15	0.011
					1799889	-675insG	157	259	140	0.49	0.099
<i>IL6</i>	3569	7p21	6p21.3	7124	—	—	—	—	—	—	
Tumor necrosis factor alpha[38,39]	<i>TNFα</i>	7124	6p21.3	—	—	—	—	—	—	—	
Serine proteinase inhibitor 1 [40]	<i>SERPINE1</i>	5054	7q21.3-22	—	—	—	—	—	—	—	

The table is based on those obese individuals among the 771 enrolled in the NUGENOB study who completed the dietary intervention ($n = 648$), who have been genotyped ($n = 643$) and who had a body mass index < 60 ($n = 642$). The genes are grouped according to their known or presumed function leading to the association with the obesity-related phenotypes.

^aFor SLC6A14, results are presented separately for women and men since the SNP is located on chromosome X. One male individual was identified as heterozygote on SLC6A14, and was excluded.

^bAll tests for Hardy-Weinberg equilibrium are based on the sum of the Pearson test statistics for each centre. The p -value is calculated from a χ^2 distribution with 8 degrees of freedom.

SNP, single nucleotide polymorphism; Rs, Reference SNP accession ID.

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comparing with a χ^2 distribution with 8 degrees of freedom. Genotype distributions in various subgroups (eligible versus noneligible, women versus men, randomised diet groups, and completers versus noncompleters of the intervention) were compared by the Pearson χ^2 statistics as appropriate. The primary outcome in the present analysis was mean WL (in kg) with 95% confidence intervals (CI), conditional on genotype and assigned diet. The WL was calculated as the difference between weight recorded immediately before randomisation and the weight at the completion of the intervention programme. Differences in body weight changes were compared by regression models, in which we controlled for centre (Gaussian random effect), gender, age (linear and squared), and baseline weight (linear) separately for men and women. The distributions of the residuals were compatible with the normal distribution and this was verified by the Shapiro-Wilk test.

The regression model addressing the effects of the genotypes on WL—main effects assessed in one model for each genotype—included the genotypes for each variant as covariates and as a separate covariate, the diet group to which the participants had been randomised. The gene-diet interaction analysis was carried out by estimating for each genotype the difference in WL, adjusted by the regression model as described, between the low-fat group (lf) and the high-fat (hf) group, and then comparing the differences in WL by diet group for a particular genetic variant (ga) and the noncarrier (gn); using the indicated notation, the outcome variable expressing the interaction would be derived from this equation: [WL(lf, ga) – WL(hf, ga)] – [WL(lf, gn) – WL(hf, gn)]. Hence, a positive value of an estimated WL difference indicates a greater WL on the low-fat diet than on the high-fat diet in individuals carrying the particular gene variant than in noncarriers of the variant.

For all the genes in which genotyping was carried out for more than one SNP (see Table 2), haplotype-based analysis was carried out by the programme GENECOUNTING (version 1.3) [57], which implements an Expectation Maximisation algorithm for estimation of the haplotype probabilities in unrelated participants without reference to the phenotype. Subsequently, we estimated the relation between the possible haplotypes on each chromosome and WL by using haplotype pairs for each participant as covariates in regression models with WL as outcome. This was carried out by multiple imputations. In each of 1,000 imputations, a new dataset was generated where the haplotype pairs for each participant were drawn at random according to the haplotype probabilities for all the possible haplotypes for each individual as estimated by the GENECOUNTING programme. The imputed datasets are each analysed by the same univariate model; the WL differences and its variances were estimated based on the method of imputation, and a test for departure from no association was constructed [58]. This approach exploits the entire information on possible haplotypes for each participant in contrast to conventional methods selecting for each individual only the most probable haplotype, implying that all other less likely haplotypes are discarded.

The statistical significance was set at $p < 0.05$, but as mentioned above, the interpretation of the significance level must take into account that testing is performed for two different types of hypotheses (gene effects on WL as such, and

gene \times diet interaction), four different types of genetic models (unspecified, dominant, co-dominant, and recessive) and 42 different SNPs (i.e., in $2 \times 168 = 336$ partially dependent analyses). Although no adequate formal correction for multiple testing is available for this scenario, any apparently statistically significant outcome of single tests must be considered tentative, requiring independent replication in other studies.

The statistical software SPSS version 11.5 (SPSS, Chicago, Illinois, United States); SAS version 8.2 (SAS Institute, Cary, North Carolina, United States); and STATA version 8.0 (Stata, College Station, Texas, United States), were used as appropriate.

RESULTS

Patient Flow and Numbers Analysed

The outcome of the dietary intervention trial has been reported in detail elsewhere [13]. Out of the 771 obese individuals enrolled in the trial, 648 completed the dietary intervention, and genotype data were available in 643 of these participants because of lack of DNA or genotyping failure in 5 participants.

In this group, 129 individuals (20.1%) exhibited characteristics deviating from the recruitment criteria, predominantly because of lower baseline BMI (range 26.4–29.9, mean 29.2) in 35 individuals (5.5%) or weight instability during the last 3 mo before enrolment in 73 (11.4%), but they were all kept in the present analysis because there were no prior reasons to assume that this would affect the results of the analyses. One participant was considered an outlier by having a combination of an excessively great BMI (66.1 kg/m²; all others were below or equal to 56 kg/m²) and increase in body weight during the intervention, which affected the underlying basic regression model. Therefore, the present analyses were based on 642 individuals. The relation between the originally recruited 771 participants and the distribution on the randomised groups are shown in Figure 1.

Baseline Data

There were only minor differences in the distributions of genotypes between this group and those who did not complete the intervention (unpublished data). The distribution of the genotypes on noncarriers, heterozygotes and homozygotes, the allele frequencies, and the outcome of testing for Hardy-Weinberg equilibrium are shown in Table 2. All allele frequencies exceeded 0.05 except for *GSHR* –447 C>G, which, however, was retained in the analysis. As expected, it appears that the frequencies of homozygotes for a number of alleles were so small that analysis of associations with specific genetic models would be impossible or very uncertain as also indicated in the estimated least detectable effects above. The global test for Hardy-Weinberg equilibrium of all genotypes showed no significant departures within each centre. As seen in Table 2, the test for each individual genotype showed that all SNPs barring three (*GAD2*, +83897 T>A; *ADIPOQ*, +276 T>G; and *TNF α* , –308 G>A) were in Hardy-Weinberg equilibrium, but in view of the unsuspecting and minor departure from the equilibrium, these SNPs were kept in the analysis. The genotype distributions showed no or small differences (none of which was statistically significant after appropriate adjustment for

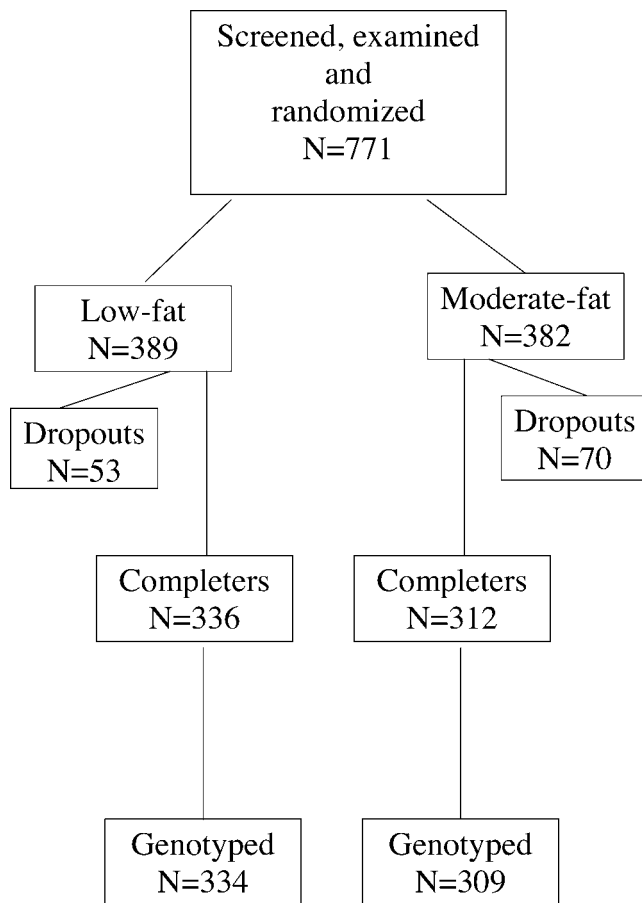


Figure 1. Patient Flow in the Trial
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multiple testing), between men and women, between the two diet groups, or between the (in retrospect) eligible and noneligible groups (unpublished data), and it was therefore not considered necessary when testing each of the genotypes to adjust for the other genotypes.

Total energy intake and dietary fat energy percent during the interventions were within the targeted intervals (Table 1). Mean WL was 6.9 kg in the low-fat group, and 6.7 kg in the high-fat group, with no significant group difference (mean 0.3 [95% CI, -0.3 to 0.8] kg), and this corresponds closely to the WL expected from the dietary energy restriction. The two diet groups did not differ in baseline and decrease in fat body mass, lean body mass, waist circumference, hip circumference and fasting insulin, nor in glucose levels [13]. The blood-lipid profiles differed as expected from the differences in diet composition (the low-fat diet group had greater decline in plasma cholesterol, plasma low-density lipoprotein cholesterol, and plasma high-density lipoprotein cholesterol, and less decline in plasma triacylglycerol), which supports that the dietary intervention did achieve the desired difference in the diet consumed [13]. There was, however, a considerable unexplained interindividual variation in WL within both groups, with standard deviations of 3.4 kg and 3.5 kg in the low-fat and high-fat groups, respectively (Table 1), which could be caused by genetic differences.

Outcomes and Estimation

Genetic effects on WL. Table 3 shows the results of the analysis of effects of genotypes on WL while controlling for the diet group (main effects of genotypes) without specifying genetic models. The table presents the difference in WL between the groups of participants who are heterozygous and homozygous for the risk SNPs and those who are homozygous noncarriers of the risk alleles, which thus is the reference group and therefore not depicted. None of the genotypes had statistically significant influence on WL in general. The effects in heterozygotes, compared with noncarriers, ranged from -0.6 to 0.8 kg, and all the CIs overlapped or included 0.0 kg. The effects in homozygotes ranged from -0.7 to 3.1 kg, and all CIs overlapped 0.0 kg. All analyses of the general effects of the haplotypes on WL were nonsignificant (unpublished data), except for those of *ENPPI* ($p = 0.04$), see below.

Gene-diet interaction effects on WL. Table 4 shows the outcome of the analyses of effects of gene-diet interaction on WL, i.e., the possible effects of genotype on the efficacy of the particular dietary intervention. If there were no interaction between the particular genotype and the dietary intervention, then the differences in WL between the dietary groups for each genotype would be the same, and the figures in the table would be 0.0 kg, whereas a value for the estimated WL significantly departing from 0.0 kg would indicate a gene-diet interaction. Positive figures mean that the WL is greater on the low-fat than with the high-fat diet when the risk SNP is present than when it is not present (noncarrier), and vice-versa for negative figures. As in Table 3, those who are noncarriers of the risk SNPs are the reference groups and thus not depicted.

The genotype-dependent excess WL on low-fat diet compared with high-fat diet ranged from 1.9 to -1.6 kg in heterozygotes, and from 3.8 kg to -2.1 kg in homozygotes, but, except for association with one SNP in *ADIPOQ* (-11377 C>G) and the SNP of the *TNFA*, none were significant. These two test results would not be statistically significant if the multiple testing related to Table 4 was taken into account.

None of the haplotype analyses showed any overall statistically significant effects, and judged from the CI, none of the individual haplotypes was statistically significant.

Ancillary Analyses

Specific genetic models and specific haplotypes. In single tests significant association ($p < 0.05$) with WL was found for the SNPs of the genes *PCSK1*, *WAC*, *HSD11B1*, and *TNFA*, when assuming specific genetic models, and these findings are summarised in Table 5.

For the *GAD2* gene, the global analysis of the haplotypes was not significant, but there was one haplotype, composed of a combination of all three risk SNPs, which compared with the haplotype without any risk SNPs, showed a borderline effect according to the CI (Table 5). The effect of the haplotype in which only the *ENPPI* SNP 1044 A>G was present with the other loci without the risk SNPs appeared to be the main contributor to the significant effect compared with the haplotype including none of the risk SNPs (Table 5).

When assuming specific genetic models in the analyses of the dietary interactions with the genotypes, we found few statistically significant ($p < 0.05$) interaction effects for

Table 3. Differences in Weight Loss (WL) by Genotypes (with the Noncarrier Reference) following 10-wk Dietary Intervention, Aiming at Reducing the Energy Intake by 600 kcal in 642 European Obese Women and Men

Gene	Single Nucleotide Polymorphism (SNP)	Heterozygous		Homozygous		Single SNP Test	Haplotype Test ^b
		WL ^a	CI	WL ^a	CI	p	p
<i>SLC6A14</i> Women	+22510 C>G	0.1	(-0.7,0.9)	-0.1	(-0.9,0.8)	0.90	
<i>SLC6A14</i> Men	+22510 C>G	—	—	-0.7	(-1.9,0.5)	0.27	
<i>CART</i>	-3608 T>C	0.5	(-0.1,1.2)	-0.1	(-0.8,0.6)	0.09	0.91
	-1702 C>T	0.5	(-0.1,1.1)	-0.2	(-0.9,0.5)	0.07	
	-175 A>G	0.5	(-0.1,1.2)	0.0	(-0.7,0.7)	0.09	
	-1336 delA	-0.6	(-1.3,0.1)	1.9	(-0.6,4.5)	0.06	
<i>GHSR</i>	-447 C>G	-0.1	(-1.3,1.1)	—	—	0.87	
<i>GAD2</i>	+83897 T>A	-0.5	(-1.0,0.1)	-0.4	(-1.5,0.8)	0.27	0.33
	+61450 C>A	-0.1	(-0.6,0.4)	-0.5	(-1.4,0.4)	0.56	
	-243 A>G	-0.3	(-0.9,0.3)	-0.6	(-1.8,0.6)	0.40	
<i>GHRL</i>	Leu72Met	0.3	(-0.5,1.1)	-0.7	(-3.9,2.4)	0.64	
<i>MKKS</i>	Arg517Cys	0.6	(-0.1,1.2)	1.1	(-5.1,7.3)	0.22	
<i>LEPROTL1</i>	-2625 C>T	0.5	(-0.3,1.4)	-0.2	(-6.4,6.1)	0.49	
<i>PCSK1</i>	T6905 C>G	-0.5	(-1.1,0.0)	-0.5	(-1.4,0.5)	0.14	
<i>UCP2</i>	-866 G>A	-0.3	(-0.8,0.2)	0.0	(-0.8,0.8)	0.45	
<i>UCP3</i>	-55 C>T	0.0	(-0.5,0.6)	0.5	(-0.4,1.5)	0.56	
<i>FOXC2</i>	-512 C>T	0.2	(-0.6,1.0)	0.3	(-0.4,1.1)	0.68	
<i>PPARG1A</i>	Gly482Ser	0.7	(-0.2,1.5)	0.3	(-0.5,1.1)	0.23	0.14
	+2962A/G	0.5	(-0.1,1.0)	0.6	(-0.1,1.3)	0.18	
<i>PPARG2</i>	Pro12Ala	0.0	(-0.6,0.6)	0.5	(-1.4,2.3)	0.87	0.13
	1431 C>T	-0.3	(-0.9,0.3)	-0.4	(-2.3,1.5)	0.59	
	-820 C>T	0.0	(-0.6,0.6)	0.1	(-1.7,1.8)	1.00	
<i>PPARG3</i>	-681C>G	-0.5	(-1.0,0.0)	0.1	(-1.0,1.2)	0.14	
<i>SREBF1</i>	17 C>G	0.0	(-0.6,0.5)	-0.1	(-0.9,0.7)	0.96	
<i>WAC</i>	-829 A>G	-0.3	(-0.9,0.4)	0.4	(-0.3,1.2)	0.09	
<i>HSD11B1</i>	-2940 G>A	0.8	(0.0,1.6)	3.1	(-3.1,9.4)	0.11	
<i>LIPC</i>	644 A>G	0.2	(-0.3,0.8)	0.5	(-0.4,1.3)	0.48	0.38
	-557 C>T	-0.2	(-0.8,0.3)	0.9	(-0.5,2.3)	0.27	
<i>IGF2</i>	1926 C>G	0.1	(-0.4,0.6)	0.5	(-0.4,1.4)	0.54	0.73
	Apal A>G	0.1	(-0.4,0.7)	0.8	(-0.2,1.7)	0.26	
	-6815 A>T	0.0	(-0.5,0.5)	0.5	(-0.5,1.4)	0.60	
<i>KCNJ11</i>	Glu23Lys	-0.5	(-1.0,0.1)	0.3	(-0.6,1.1)	0.09	
<i>ENPP1</i>	IVS20delT-11	-0.3	(-0.9,0.2)	0.0	(-1.2,1.3)	0.45	0.04
	Lys121Gln	0.2	(-0.3,0.8)	1.3	(-0.6,3.1)	0.33	
	1044 A>G	0.4	(-0.1,1.0)	0.6	(-0.4,1.7)	0.21	
<i>ADIPOQ</i>	-11377 C>G	0.1	(-0.4,0.6)	0.2	(-0.9,1.3)	0.89	0.50
	+45 T>G	0.1	(-0.5,0.8)	-0.6	(-2.0,0.8)	0.62	
	+276 T>G	-0.3	(-1.1,0.6)	0.0	(-0.9,0.8)	0.67	
	-11391 G>A	0.2	(-0.5,0.8)	0.5	(-3.1,4.1)	0.88	
<i>CD36</i>	-178 A>C	0.4	(-0.3,1.1)	0.5	(-0.3,1.2)	0.43	
<i>IL6</i>	-174 G>C	0.0	(-0.6,0.5)	0.0	(-0.8,0.7)	0.99	
<i>TNFα</i>	-308 G>A	0.6	(0.0,1.1)	0.9	(-0.8,2.6)	0.13	
<i>SERPINE1</i>	-675insG	-0.1	(-0.7,0.5)	-0.3	(-1.0,0.4)	0.70	

WL (in kg) is adjusted for gender, diet group, age (linear and squared), baseline weight (separate effect for men and women), and centre (Gaussian random effect).

^aA positive value of the estimated WL difference indicates a greater WL in individuals carrying the particular SNP than in noncarriers of the gene.

^bThe test for association between the haplotypes of the genes where more than one SNP were genotyped is carried out as a global test for the possible haplotypes (see Methods section for details).

CI, confidence intervals; SNP, single nucleotide polymorphism.

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variants in the *SLC6A14*, *KCNJ11*, *ENPP1* (1044 A>G), *ADIPOQ* (-11391 G>A) and *TNF α* genes (Table 5).

None of the results shown in Table 5 was adjusted for multiple testing effects, which would have made all results statistically insignificant.

Adverse Events

As reported previously, there were no adverse events [13]. Dropouts were because of changes in personal circumstances, dislike of the diets, and emerging health problems unrelated to the diets.

DISCUSSION

Interpretation

This study aimed at utilising the randomised dietary intervention as an experimental approach to investigate whether the effects of a panel of 42 SNPs in 26 candidate genes are associated with diet-induced WL, and whether these effects are sensitive to the fat (versus the carbohydrate) content of a hypo-energetic diet. All the genes are known or presumed to be associated with obesity-related phenotypes or to be involved in pathophysiological processes implicated in obesity. The overall conclusion from the analyses is that there

Table 4. Effects of Genotype–Diet Interactions on Weight Loss (WL) following 10-week Dietary Intervention, Aiming at Reducing the Energy Intake by 600 kcal Either by a Low-Fat or a High-Fat Diet Allocated at Random to 642 European Obese Women and Men

Gene	Single Nucleotide Polymorphism (SNP)	Heterozygous		Homozygous		Single SNP Test	Haplotype Test ^c
		WL ^b	CI	WL ^b	CI	p	p
<i>SLC6A14</i> Women ^a	+22510 C>G	1.9	(0.3; 3.5)	1.6	(−0.1; 3.2)	0.06	
<i>SLC6A14</i> Men ^a	+22510 C>G	—	—	0.3	(−2.0; 2.7)	0.78	
<i>CART</i>	−3608 T>C	−0.9	(−2.1; 0.3)	−0.5	(−1.9; 0.9)	0.36	0.66
	−1702 C>T	−1.0	(−2.1; 0.1)	0.2	(−1.2; 1.6)	0.10	
	−175 A>G	−0.5	(−1.7; 0.8)	−0.5	(−1.9; 0.9)	0.72	
	−1336 delA	0.6	(−0.8; 1.9)	1.0	(−4.4; 6.5)	0.68	
<i>GHSR</i>	−447 C>G	1.1	(−1.3; 3.6)	—	—	0.35	
<i>GAD2</i>	+83897 T>A	0.6	(−0.5; 1.7)	−0.2	(−2.6; 2.1)	0.56	0.80
	+61450 C>A	0.1	(−1.0; 1.1)	−0.6	(−2.4; 1.2)	0.78	
	−243 A>G	0.1	(−1.0; 1.3)	0.6	(−1.9; 3.0)	0.88	
<i>GHRL</i>	Leu72Met	0.8	(−0.8; 2.4)	3.8	(−2.5; 10.1)	0.33	
<i>MKKS</i>	Arg517Cys	0.5	(−0.8; 1.7)	—	—	0.47	
<i>LEPROTL1</i>	−2625 C>T	1.4	(−0.4; 3.2)	—	—	0.12	
<i>PCSK1</i>	T6905 C>G	0.4	(−0.7; 1.4)	0.5	(−1.5; 2.4)	0.76	
<i>UCP2</i>	−866 G>A	−0.8	(−1.9; 0.3)	0.2	(−1.4; 1.8)	0.25	
<i>UCP3</i>	−55 C>T	−0.2	(−1.3; 0.8)	0.2	(−1.8; 2.2)	0.86	
<i>FOXC2</i>	−512 C>T	−0.2	(−1.7; 1.4)	−0.5	(−2.1; 1.1)	0.75	
<i>PPARG1A</i>	Gly482Ser	−1.2	(−2.8; 0.5)	−1.0	(−2.6; 0.7)	0.39	0.23
	+2962A/G	−0.2	(−1.4; 1.0)	0.7	(−0.7; 2.1)	0.36	
<i>PPARG2</i>	Pro12Ala	0.3	(−0.9; 1.5)	0.1	(−3.8; 4.1)	0.88	0.94
	1431C>T	0.6	(−0.6; 1.8)	−1.3	(−5.2; 2.5)	0.45	
	−820 C>T	0.3	(−0.9; 1.5)	−0.2	(−4.0; 3.5)	0.87	
<i>PPARG3</i>	−681C>G	−0.3	(−1.4; 0.7)	0.3	(−1.9; 2.5)	0.76	0.94
<i>SREBF1</i>	17 C>G	−0.2	(−1.2; 0.9)	−1.4	(−3.0; 0.2)	0.20	
<i>WAC</i>	−829 A>G	−1.2	(−2.5; 0.1)	−1.2	(−2.6; 0.3)	0.17	
<i>HSD11B1</i>	−2940 G>A	0.6	(−1.0; 2.1)	—	—	0.49	
<i>LIPC</i>	644 A>G	−0.7	(−1.8; 0.4)	−0.3	(−2.0; 1.4)	0.42	0.58
	−557 C>T	−0.4	(−1.5; 0.6)	−0.8	(−3.7; 2.1)	0.67	
<i>IGF2</i>	1926 C>G	−0.8	(−1.9; 0.3)	−0.2	(−2.0; 1.6)	0.34	0.41
	Apal A>G	−0.6	(−1.6; 0.5)	−0.3	(−2.1; 1.5)	0.59	
	−6815 A>T	−0.1	(−1.1; 1.0)	0.6	(−1.3; 2.5)	0.77	
<i>KCNJ11</i>	Glu23Lys	0.7	(0.3; 1.8)	1.7	(0.1; 3.4)	0.10	
<i>ENPP1</i>	IVS20 del T −11	−0.5	(−1.5; 0.5)	−0.6	(−3.1; 2.0)	0.62	0.55
	Lys121Gln	−0.2	(−1.4; 0.9)	−0.9	(−4.5; 2.8)	0.85	
	1044 A>G	0.1	(−1.0; 1.2)	−2.1	(−4.2; 0.0)	0.13	
<i>ADIPOQ</i>	−11377 C>G	1.2	(0.2; 2.3)	−1.0	(−3.1; 1.2)	0.029	0.43
	+45 T>G	−1.1	(−2.4; 0.1)	0.6	(−2.2; 3.4)	0.18	
	+276 T>G	1.5	(−0.1; 3.2)	0.8	(−0.8; 2.4)	0.14	
	−11391 G>A	−1.6	(−3.0; −0.2)	−0.9	(−8.6; 6.8)	0.07	
<i>CD36</i>	−178 A>C	0.4	(−1.0; 1.8)	1.0	(−0.5; 2.5)	0.42	
<i>IL6</i>	−174 G>C	0.6	(−0.6; 1.8)	0.4	(−1.1; 1.9)	0.60	
<i>TNFα</i>	−308 G>A	1.5	(0.3; 2.6)	−0.1	(−3.8; 3.6)	0.04	
<i>SERPINE1</i>	−675 insG	0.8	(−0.5; 2.0)	−0.1	(−1.6; 1.3)	0.29	

WL (in kg) adjusted for gender, age (linear and squared), baseline weight (separate effect for men and women), and centre (Gaussian random effect). The WL is the difference in excess WL by low-fat diet compared with high-fat diet between individuals with the particular genotype and noncarrier individuals.

^a*SLC6A14* results are presented separately for men and women since the SNP is located on chromosome X.

^bA positive value of the estimated WL difference indicates a greater WL on the low-fat diet than on the high-fat diet in individuals carrying the particular SNP than in noncarriers of the gene.

^cThe test for association between the haplotypes of the genes where more than one SNP were genotyped is carried out as a global test for the possible haplotypes (see Methods section for details).

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are no indications in these results of a major influence of any of these genetic polymorphisms on the clinical outcome of the intervention. When considering the several dimensions of multiple testing carried out to address this subject, the few statistically significant results, obtained without any adjustment for the multiple testing, should only be considered as leads to new hypotheses about effects of specific genetic polymorphisms that need to be addressed in subsequent studies. Thus, the study suggests that SNPs in the *PCSK1*, *WAC*, *HSD11B1*, and *TNFα*, genes and possibly haplotypes of the *GAD2* and *ENPP1* genes may modulate diet-induced

weight changes, and that the effect of dietary fat content may depend on SNPs in the *SLC6A14*, *KCNJ11*, *ENPP1*, *ADIPOQ*, and *TNFα* genes.

The problem of multiple testing of the genotype-phenotype relationships has no unambiguous quantitative solution. On one hand, there are clearly elements of testing of null hypotheses that could not be distinguished on prior reasons that make it unjustifiable to consider each single statistical test independently of all the others. On the other hand, it may not be justified to consider all tests as tests of the same null hypothesis, in which case conventional quantitative adjust-

Table 5. Statistically Significant Associations (without Adjustment for Multiple Testing Effects)

Gene	SNP	Diet Interaction	Genetic Model	WL	CI	p
<i>SLC6A14</i> Women	+22510 C>G	Yes	Dominant	1.8	(0.3; 3.3)	0.019
<i>GAD2</i>	+83897 T>A, Ca +61450 C>A, Ca -243 A>G, Ca	No	Haplotype ^a	-0.4	(-0.9; 0.0)	0.33 ^b
<i>PCSK1</i>	T6905 C>G	No	Dominant	-0.5	(-1.0; 0.0)	0.048
<i>WAC</i>	-829a A>G	No	Recessive	-0.6	(-1.2; 0.0)	0.043
<i>HSD11B1</i>	G>A	No	Co-dominant	0.8	(0.0; 1.6)	0.039
	G>A	No	Dominant	0.8	(0.0; 1.6)	0.047
<i>KCNJ11</i>	Glu23Lys	Yes	Co-dominant	0.8	(0.1; 1.6)	0.032
<i>ENPP1</i>	IVS20delT-11, Nca Lys121Gln, NCa 1044 A>G, Ca	No	Haplotype ^a	0.8	(0.1; 1.5)	0.04
<i>ENPP1</i>	1044 A>G	Yes	Recessive	-2.1	(-4.1; 0.0)	0.045
<i>ADIPOQ</i>	-11391 G>A	Yes	Co-dominant	-1.6	(-2.9; -0.2)	0.027
	-11391 G>A	Yes	Dominant	-1.6	(-2.9; -0.2)	0.023
<i>TNFα</i>	-308 G>A	No	Co-dominant	0.5	(0.0; 1.0)	0.046
	-308 G>A	No	Dominant	0.6	(0.0; 1.2)	0.048
	-308 G>A	Yes	Co-dominant	1.0	(0.0; 2.0)	0.047
	-308 G>A	Yes	Dominant	1.4	(0.3; 2.5)	0.015

Among particular genetic models (dominant, co-dominant, or recessive with regard to the effects of the single nucleotide polymorphisms) or haplotypes and weight loss (WL), independent or dependent of interaction with the allocated diet in a 10-wk dietary intervention, aiming at reducing the energy intake by 600 kcal either by a low-fat or a high-fat diet allocated at random to 642 European obese women and men.

WL (in kg) is adjusted for gender, diet group, age (linear and squared), baseline weight (separate effect for men and women), and centre (Gaussian random effect). A positive value of the estimated difference in WL indicates a greater WL in individuals carrying the particular SNP or haplotype than in noncarriers. When there is an interaction with the diet, then the WL is the difference in excess WL by low-fat diet compared with high-fat diet between individuals with the particular genotype and those not carrying the genotype.

^aThe haplotypes listed are those indicated with Ca and Nca for carrier and noncarrier of the listed polymorphisms, and they were compared with a reference group of individuals who had haplotypes not including any of the listed polymorphisms (see Methods section for details).

^bFor the *GAD2* haplotypes, there were no global statistically significant association, but the listed haplotype showed confidence limits not overlapping zero, wherefore it is included in the table.

SNP, single nucleotide polymorphism.

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ment by, for example, the Bonferroni method would be justified. However, the number of statistically significant tests at $p = 0.05$ was about what would be expected under repetitive testing of the same null hypothesis. This suggests that any of the statistically significant findings must be cautiously interpreted and only used as basis for new studies of the possible role of these genetic polymorphisms.

The present study has limitations regarding both the genotypes and the phenotypes. The analysis of SNPs in the candidate genes took place without control of the possibly associated variation in the genomic context. Despite the control for population origin by centre, there may still be population stratification that can produce false effects or obscure the true effects. Moreover, any findings of genotype effects may reflect that the particular SNP is in linkage disequilibrium with other SNPs within the locus or even in the neighbouring chromosomal region, extending beyond the haplotypes of the particular genes tested; and these other SNPs may, in fact, be responsible for the observed effects. The criteria for selection of the SNPs were not based on prior evidence suggesting a specific functional role in dietary-induced WL, which was not available. For several SNPs, the homozygotes were so uncommon that estimates of recessive effects or distinction between dominant and co-dominant effects were impossible, which is also reflected in the width of the CIs. For these SNPs, the analytical focus is on the general nonspecified and/or the dominant genetic models. The speculative possibility that the effect of the SNPs in one gene may depend on genetic sex differences or on the presence of particular SNPs in another gene (gene-gene interactions or

epistasis) could not be adequately addressed in the present study, but the genotype distributions in men and women and in the two randomised diet groups were almost similar. Finally, the selection of SNPs in the candidate genes may not provide a comprehensive coverage of the steadily evolving knowledge about the genetic variation in these genes, so we cannot exclude the possibility that other SNPs, not in linkage disequilibrium with those investigated, in some of the genes may have an influence on the clinical outcomes.

Not all individuals enrolled in the NUGENOB trial completed the dietary intervention or strictly met the recruitment criteria, but previous analysis, using imputation of the missing values and sensitivity analysis have shown that the overall results of the two diets on WL are quite robust [13]. Moreover, the genotype distributions were almost similar in these subgroups. We therefore find it unlikely that the inclusion of the participants not fulfilling the recruitment criteria or the restriction of the present study to those completing the dietary intervention has caused any bias in assessment of the role of the genetic variation on WL. On average, targets set for dietary changes were achieved, as also verified by the average WL and the diet-dependent differences in changes in blood lipids [13], but there were considerable individual differences in reported dietary intake, both with regard to energy restriction and dietary composition (see Table 2). It is possible that differences in actual intake have obscured some main effects or interaction effects of the SNPs on the WL, but the likely measurement errors in reported dietary intake prohibit a valid search for such effects. The study period is limited to 10 wk, and the

energy restriction was moderate, so it is possible that the more prominent gene-diet interactions may emerge by more severe energy restriction (e.g., by very-low-calorie diets), or during more prolonged intervention and weight maintenance diet. On the other hand, current management strategies for obesity do aim at rather moderate WL as clinically relevant goals [59,60]. Furthermore, it is possible that interactions between genes and diet composition may depend on whether or not there is a concomitant dietary energy restriction. The phenotype analysed is a composite phenotype, and although the average changes in anthropometrics and body composition were similar in the two diet groups [13], the genotype effects on the reduction of adipose tissue mass at various sites (i.e., general versus peripheral and abdominal sites) may be different. The study size did not allow a more detailed analysis of differential effects of the genotypes by differences in other characteristics, including those adjusted for in the analysis, (e.g., gender, age and baseline body weight).

Generalisability

The study population originates from various parts of Europe that have different obesity prevalence rates. It can thus be assumed to be rather heterogeneous with respect to the genetic and/or the environmental factors that may influence body weight regulation. Analysis of the allele distribution of the genes tested in the present study has shown that there is great genetic heterogeneity between the populations from the various participating sites (C. Dina et al., unpublished data). We adjusted the analysis (using random effects models) for confounding because of such differences, which, however, assumes that the gene-diet interaction as such is not modified by factors that differ among the various parts of Europe.

The fairly strict inclusion criteria as well as the recruitment basis and procedures may have implications for the generalisability of the results. One obvious such limitation is illustrated by the predominance of women in the trial. On the other hand, this and other aspects of the characteristics of the participants in the trial are likely to represent the patient population seeking dietary treatment for their obesity problem. We have no prior reasons to believe that any of the constraints on the study population would modify the results of the study.

Overall Evidence

In the NUGENOB trial, we found almost equal mean WL in the two diet groups, but there was a considerable interindividual variation in WL [13], to which genetic variation may contribute. However, the observed effects of the SNPs in the genes were moderate and the findings need to be further investigated before they can be considered contributing to the individual variation in WL during dieting.

Thus, these results do not contribute evidence to future optimisation of dietary treatment of obesity by tailoring the diet to the individual patients according to genotypes that may predict the outcome of the treatment. Although the range of observed WL differences does have clinical significance [59,60], the findings in the present study do not, by themselves, lend support to such use of the genotypes in the panel of genetic polymorphisms tested. When judged to be clinically indicated, either of the dietary regimens investigated may be used for inducing WL in obese individuals in accordance with the previously reported results [13].

Most effects were compatible with dominant and/or additive co-dominant effects, and only SNPs in the *ENPP1* and *WAC* genes had recessive effects, which also were less likely to be discovered because of the lower frequency of homozygotes for the particular variant. The SNPs in the *HSD11B1*, *TNF α* , and the *WAC* genes and a haplotype of *ENPP1* increased the WL, whereas the SNPs in the *GAD2* and *PCSK1* genes reduced the WL. The SNPs in the *SLC6A14*, *KCJN11*, and *TNF α* lead to an increased WL by a low-fat diet compared to a high-fat diet, whereas SNPs of the *ENPP1* and *ADIPOQ* had the opposite effect.

The genes in which significant effects of the SNPs were observed belonged to several different categories of presumed function in the obesity-related phenotypes (see Table 2). The *SLC6A14* and *PCSK1* genes and possibly *GAD2* are presumed to influence hypothalamic regulation of appetite [15,17–19]. The *WAC* gene may be involved in regulation of adipocyte differentiation and function [38]. Several of the genes presumed to be associated with regulation of lipid and glucose metabolism (the *HSD11B1*, *IGF2*, *KCJN11*, and *ENPP1* genes) [39,42–46] and the production of adipocytokines (the *ADIPOQ* and *TNF α* genes) [47,48,52,53] were involved. Surprisingly, the SNPs in the candidate genes affecting efficiency of energy expenditure (*UCP2* and *UCP3*) showed no effects. Clearly, a better understanding of the specific roles of the SNPs of these genes in altering the function of the multiple, possibly interacting pathways, involved in producing the WL by the particular diets, will require further exploration of how the SNPs affect the intermediate phenotypes, possibly also during overfeeding and weight gain. The differences in effects on WL and the statistical strength of the various SNPs were rather small, so the impact of the SNPs on dietary-induced WL may primarily depend on the frequency of the variant allele, which was highest for the *WAC*, *SLC6A14*, and *KCJN11* genes, which, for this reason, may deserve particular attention.

The problems in interpretation of the findings in the present study encourage considerations of how the potential of the modern access to the genomic information may be used in the future in such clinical studies, as also exemplified in a recent large-scale study of candidate genes associations to type 2 diabetes [61]. In addition to the obvious request of greater sample sizes, possibly by replication of the study (which may be very challenging and expensive) it should be considered to reduce the statistical noise in the assessments of the correlations and associations between genotypes and phenotypes. A variety of approaches may be employed, including refined definitions of genotypes and phenotypes, better control of background genetic and environmental factors also influencing the phenotype at interest, better study designs and methods for measurements of phenotypes, and further developed statistical tools for handling multiple testing. Identification of intermediate phenotypes closer to the pathway steps to the genes combined with better knowledge about both within and between locus interactions of the genetic variants may both reduce the statistical noise and yield a deeper insight into how the genes operate and how the genetic variation eventually influences the clinically relevant phenotype.

In conclusion, the results of the present study suggest that polymorphisms in several genes associated with obesity-related phenotypes did not have any major impact on weight

reduction during short-term either high-fat or low-fat hypo-energetic diet in obese participants, as implemented in the present study. Thus, the results as such do not provide evidence to support revision of current dietary treatment regimens for obesity on the basis of individual genotyping. The results gave tentative leads that some genetic polymorphisms may modulate the diet-induced WL, but this needs to be confirmed and further explored in future studies.

SUPPORTING INFORMATION

CONSORT Statement

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Trial Protocol

Found at DOI: 10.1371/journal.pctr.0010012.sd002 (567 KB PDF).

Annexes to the Trial Protocol

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Alternative Language Abstract S1. Translation of the abstract into Czech.

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Alternative Language Abstract S2. Translation of the abstract into Danish.

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Alternative Language Abstract S3. Translation of the abstract into Dutch.

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Alternative Language Abstract S4. Translation of the abstract into French.

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Alternative Language Abstract S5. Translation of the abstract into Spanish.

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Alternative Language Abstract S6. Translation of the abstract into Swedish.

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REFERENCES

1. Maes HH, Neale MC, Eaves LJ (1997) Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet* 27: 325–351.
2. Bell CG, Walley AJ, Froguel P (2005) The genetics of human obesity. *Nat Rev Genet* 6: 221–234.
3. Verdich C, Clément K, Sørensen T (2004) Nutrient-gene interactions in the control of obesity. In Remacle C, Reusens B, eds. *Functional foods, ageing and degenerative disease*. Cambridge: Woodhead Publishing Ltd. 223–259.
4. Perusse L, Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, et al. (2005) The human obesity gene map: the 2004 update. *Obes Res* 13: 381–490.
5. Viguerie N, Vidal H, Arner P, Holst C, Verdich C, et al. (2005) Adipose tissue gene expression in obese subjects during low-fat and high-fat hypocaloric diets. *Diabetologia* 48: 123–131.
6. Dahlman I, Linder K, Arvidsson NE, Andersson I, Liden J, et al. (2005) Changes in adipose tissue gene expression with energy-restricted diets in obese women. *Am J Clin Nutr* 81: 1275–1285.
7. Loos RJ, Rankinen T (2005) Gene-diet interactions on body weight changes. *J Am Diet Assoc* 105: S29–S34.
8. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, et al. (1990) The response to long-term overfeeding in identical twins. *N Engl J Med* 322: 1477–1482.
9. Hainer V, Stunkard AJ, Kunesova M, Parizkova J, Stich V, et al. (2000) Intra-pair resemblance in very low calorie diet-induced weight loss in female obese identical twins. *Int J Obes Relat Metab Disord* 24: 1051–1057.
10. Larsen LH, Echwald SM, Sørensen TIA, Andersen T, Wulff BS, et al. (2005) Prevalence of mutations and functional analyses of melanocortin 4 receptor variants identified among 750 men with juvenile-onset obesity. *J Clin Endocrinol Metab* 90: 219–224.
11. Potoczna N, Branson R, Kral JG, Picc G, Steffen R, et al. (2004) Gene variants and binge eating as predictors of comorbidity and outcome of treatment in severe obesity. *J Gastrointest Surg* 8: 971–981.

12. Keski-Rahkonen A, Neale BM, Bulik CM, Pietilainen KH, Rose RJ, et al. (2005) Intentional weight loss in young adults: sex-specific genetic and environmental effects. *Obes Res* 13: 745–753.
13. Petersen M, Taylor MA, Saris WHM, Verdich C, Toubro S, et al. (2006) Randomized, multi-center trial of two hypo-energetic diets in obese subjects: high versus low fat content. *Int J Obes (Lond)* 30: 552–560.
14. Austin MA, Ordovas JM, Eckfeldt JH, Tracy R, Boerwinkle E, et al. (1996) Guidelines of the National Heart, Lung, and Blood Institute Working Group on Blood Drawing, Processing, and Storage for Genetic Studies. *Am J Epidemiol* 144: 437–441.
15. Durand E, Boutin P, Meyre D, Charles MA, Clement K, et al. (2004) Polymorphisms in the amino acid transporter solute carrier family 6 (neurotransmitter transporter) member 14 gene contribute to polygenic obesity in French Caucasians. *Diabetes* 53: 2483–2486.
16. Guerardel A, Barat-Houari M, Vasseur F, Dina C, Vatin V, et al. (2005) Analysis of sequence variability in the CART gene in relation to obesity in a Caucasian population. *BMC Genet* 6: 19.
17. Baessler A, Hasinoff MJ, Fischer M, Reinhard W, Sonnenberg GE, et al. (2005) Genetic linkage and association of the growth hormone secretagogue receptor (ghrelin receptor) gene in human obesity. *Diabetes* 54: 259–267.
18. Wang HJ, Geller F, Dempfle A, Schauble N, Friedel S, et al. (2004) Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J Clin Endocrinol Metab* 89: 157–162.
19. Boutin P, Dina C, Vasseur F, Dubois S, Corset L, et al. (2003) GAD2 on chromosome 10p12 is a candidate gene for human obesity. *PLoS Biol* 1: e68. DOI: 10.1371/journal.pbio.0000068
20. Korbonits M, Gueorguiev M, O'Grady E, Lecoer C, Swan DC, et al. (2002) A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children. *J Clin Endocrinol Metab* 87: 4005–4008.
21. Ukkola O, Ravussin E, Jacobson P, Perusse L, Rankinen T, et al. (2002) Role of ghrelin polymorphisms in obesity based on three different studies. *Obes Res* 10: 782–791.
22. Andersen KL, Echwald SM, Larsen LH, Hamid YH, Glumer C, et al. (2005) Variation of the McKusick-Kaufman gene and studies of relationships with common forms of obesity. *J Clin Endocrinol Metab* 90: 225–230.
23. Huang Y, Ying K, Xie Y, Zhou Z, Wang W, et al. (2001) Cloning and characterization of a novel human leptin receptor overlapping transcript-like 1 gene (LEPROTL1). *Biochim Biophys Acta* 1517: 327–331.
24. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, et al. (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 16: 303–306.
25. Esterbauer H, Schneitler C, Oberkofler H, Ebenbichler C, Paulweber B, et al. (2001) A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat Genet* 28: 178–183.
26. Dalgaard LT, Hansen T, Urhammer SA, Drivsholm T, Borch-Johnsen K, et al. (2001) The uncoupling protein 3 –55 C → T variant is not associated with Type II diabetes mellitus in Danish subjects. *Diabetologia* 44: 1065–1067.
27. Carlsson E, Almgren P, Hoffstedt J, Groop L, Ridderstrale M (2004) The FOXC2 C-512T polymorphism is associated with obesity and dyslipidemia. *Obes Res* 12: 1738–1743.
28. Yanagisawa K, Hingstrup LL, Andersen G, Drivsholm T, Cederberg A, et al. (2003) The FOXC2 –512C>T variant is associated with hypertriglyceridaemia and increased serum C-peptide in Danish Caucasian glucose-tolerant subjects. *Diabetologia* 46: 1576–1580.
29. Esterbauer H, Oberkofler H, Linnemayr V, Iglseider B, Hedegger M, et al. (2002) Peroxisome proliferator-activated receptor-gamma coactivator-1 gene locus: associations with obesity indices in middle-aged women. *Diabetes* 51: 1281–1286.
30. Ek J, Andersen G, Urhammer SA, Gaede PH, Drivsholm T, et al. (2001) Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus. *Diabetologia* 44: 2220–2226.
31. Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, et al. (1997) Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 241: 270–274.
32. Ek J, Urhammer SA, Sørensen TIA, Andersen T, Auwerx J, et al. (1999) Homozygosity of the Pro12Ala variant of the peroxisome proliferation-activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects on body mass index in obese and lean Caucasian men. *Diabetologia* 42: 892–895.
33. Doney A, Fischer B, Frew D, Cumming A, Flavell DM, et al. (2002) Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with body weight. *BMC Genet* 3: 21.
34. Valve R, Sivenius K, Miettinen R, Pihlajamaki J, Rissanen A, et al. (1999) Two polymorphisms in the peroxisome proliferator-activated receptor-gamma gene are associated with severe overweight among obese women. *J Clin Endocrinol Metab* 84: 3708–3712.
35. Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Lebel P, et al. (1998) A genetic polymorphism of the peroxisome proliferator-activated receptor gamma gene influences plasma leptin levels in obese humans. *Hum Mol Genet* 7: 435–440.
36. Meirhaeghe A, Fajas L, Gouilleux F, Cottel D, Helbecque N, et al. (2003) A functional polymorphism in a STAT5B site of the human PPAR gamma 3 gene promoter affects height and lipid metabolism in a French population. *Arterioscler Thromb Vasc Biol* 23: 289–294.
37. Eberle D, Clement K, Meyre D, Sahbatou M, Vaxillaire M, et al. (2004) SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes* 53: 2153–2157.
38. Xu GM, Arnaout MA (2002) WAC, a novel WW domain-containing adapter with a coiled-coil region, is colocalized with splicing factor SC35. *Genomics* 79: 87–94.
39. Draper N, Echwald SM, Lavery GG, Walker EA, Fraser R, et al. (2002) Association studies between microsatellite markers within the gene encoding human 11beta-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. *J Clin Endocrinol Metab* 87: 4984–4990.
40. Nair S, Lee YH, Lindsay RS, Walker BR, Tataranni PA, et al. (2004) 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. *Diabetologia* 47: 1088–1095.
41. Isaacs A, Sayed-Tabatabaei FA, Njajou OT, Witteman JC, van Duijn CM (2004) The –514 C->T hepatic lipase promoter region polymorphism and plasma lipids: a meta-analysis. *J Clin Endocrinol Metab* 89: 3858–3863.
42. Gaunt TR, Cooper JA, Miller GJ, Day IN, O'Dell SD (2001) Positive associations between single nucleotide polymorphisms in the IGF2 gene region and body mass index in adult males. *Hum Mol Genet* 10: 1491–1501.
43. Gu D, O'Dell SD, Chen XH, Miller GJ, Day IN (2002). Evidence of multiple causal sites affecting weight in the IGF2-INS-TH region of human chromosome 11. *Hum Genet* 110: 173–181.
44. Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, et al. (2003) The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52: 573–577.
45. Schwanstecher C, Meyer U, Schwanstecher M (2002) K(IR)6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic beta-cell ATP-sensitive K(+) channels. *Diabetes* 51: 875–879.
46. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoer C, et al. (2005) Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. *Nat Genet* 37: 863–867.
47. Hara K, Boutin P, Mori Y, Tobe K, Dina C, et al. (2002) Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51: 536–540.
48. Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, et al. (2002) Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet* 11: 2607–2614.
49. Lepretre F, Linton KJ, Lacquemant C, Vatin V, Samson C, et al. (2004) Genetic study of the CD36 gene in a French diabetic population. *Diabetes Metab* 30: 459–463.
50. Hamid YH, Rose CS, Urhammer SA, Glumer C, Nolsoe R, et al. (2005) Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. *Diabetologia* 48: 251–260.
51. Wernstedt I, Eriksson AL, Berndtsson A, Hoffstedt J, Skrtic S, et al. (2004) A common polymorphism in the interleukin-6 gene promoter is associated with overweight. *Int J Obes Relat Metab Disord* 28: 1272–1279.
52. Rasmussen SK, Urhammer SA, Jensen JN, Hansen T, Borch-Johnsen K, et al. (2000) The –238 and –308 G → A polymorphisms of the tumor necrosis factor alpha gene promoter are not associated with features of the insulin resistance syndrome or altered birth weight in Danish Caucasians. *J Clin Endocrinol Metab* 85: 1731–1734.
53. Brinkman BM, Zuijdeest D, Kaijzel EL, Breedveld FC, Verweij CL (1995) Relevance of the tumor necrosis factor alpha (TNF alpha) –308 promoter polymorphism in TNF alpha gene regulation. *J Inflamm* 46: 32–41.
54. Hoffstedt J, Andersson IL, Persson L, Isaksson B, Arner P (2002) The common –675 4G/5G polymorphism in the plasminogen activator inhibitor –1 gene is strongly associated with obesity. *Diabetologia* 45: 584–587.
55. Blomeke B, Shields PG (1999) Laboratory methods for the determination of genetic polymorphisms in humans. *IARC Sci Publ*: 133–47.
56. Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Heredity* 86: 248–249.
57. Zhao JH, Lissarrague S, Essioux L, Sham PC (2002) GENECOUNTING: haplotype analysis with missing genotypes. *Bioinformatics* 18: 1694–1695.
58. Rubin DB (1987) Multiple Imputation for Nonresponse in Surveys. New York: John Wiley & Sons, Inc.
59. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, et al. (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 344: 1343–1350.
60. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, et al. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346: 393–403.
61. Barroso I, Luan J, Middelberg RP, Harding AH, Franks PW, et al. (2003) Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action. *PLoS Biol* 1: e20. DOI: 10.1371/journal.pbio.0000020