Variants Near *MC4R* Are Associated With Obesity and Influence Obesity-Related Quantitative Traits in a Population of Middle-Aged People: Studies of 14,940 Danes

Dorit P. Zobel,[1] Camilla H. Andreasen,[1] Niels Grarup,[1] Hans Eiberg,[2] Thorkild I.A. Sørensen,[3] Annelli Sandbæk,[4] Torsten Lauritzen,[4] Knut Borch-Johnsen,[1,5,6] Torben Jørgensen,[5] Oluf Pedersen,[1,6,7] and Torben Hansen[1,8]

OBJECTIVE—Variants downstream of the melanocortin-4 receptor gene (*MC4R*) have been reported to associate with obesity. We examined rs17782313, rs17700633, rs12970134, rs477181, rs502933, and rs4450508 near *MC4R* for association with obesity-related quantitative traits, obesity, and type 2 diabetes in Danish individuals.

RESEARCH DESIGN AND METHODS—The variants were investigated for association with obesity-related quantitative traits in 5,807 population-based sampled individuals, obesity in 14,940 individuals, and type 2 diabetes in 8,821 individuals.

RESULTS—The minor risk alleles of rs17782313, rs17700633, and rs12970134 were associated with BMI (effect per allele 0.25 kg/m^2, $P = 0.01$; 0.23, $P = 0.01$; and 0.31, $P = 7 \times 10^{-4}$, respectively), waist circumference (0.67 cm, $P = 0.006$; 0.53, $P = 0.02$; and 0.85, $P = 3 \times 10^{-4}$), and body weight (1.04 kg, $P = 6 \times 10^{-4}$; 0.71, $P = 0.01$; and 1.16, $P = 8 \times 10^{-5}$). In case-control studies of obesity defined by BMI, the minor C-allele of rs17782313 was associated with overweight/obesity and obesity (odds ratio [OR] 1.09, $P = 0.006$ and OR 1.12, $P = 0.003$, respectively). Similarly, the minor A-allele of rs17700633 was associated with overweight/obesity and obesity (1.12, $P = 8 \times 10^{-5}$ and 1.16, $P = 2 \times 10^{-5}$), and the minor A-allele of rs12970134 was also associated with overweight/obesity and obesity (1.13, $P = 2 \times 10^{-5}$ and 1.15, $P = 6 \times 10^{-5}$). rs477181, rs502933, and rs4450508 were not significantly associated with obesity in the Danish population. The frequency of the minor risk alleles of rs17782313 and rs12970134 was higher among patients with type 2 diabetes than among glucose-tolerant individuals (OR 1.08, $P = 0.08$ and 1.08, $P = 0.06$, respectively); however, these borderline associations were abolished after adjustment for BMI.

CONCLUSIONS—rs17782313, rs17700633, and rs12970134 near *MC4R* associate with measures of obesity in Danish individuals.
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Obesity and the accompanying risk of common diseases such as type 2 diabetes and premature cardiovascular morbidity and mortality are increasing global health burdens. Multiple variations in genes are likely to contribute to the pathogenesis of obesity. Monogenic forms of obesity have been identified, with mutations in the gene encoding the melanocortin-4 receptor (*MC4R*) being the most prevalent (1–3). *MC4R* is located on chromosome 18q22 (4) and expressed in the central nervous system (5) where the encoded protein is involved in appetite regulation (6). Variation in *MC4R* has been reported to associate with common forms of obesity (7–9). Variation in the fat mass and obesity-associated gene (*FTO*) was the first example of common genetic variation for which there is widely replicated evidence of association with obesity in the general population (10–12). Recently, in a study analyzing genome-wide association data from white Europeans informative for BMI, variation in *FTO* was also found with the strongest BMI-association signal, followed by signals mapping to chromosome 18q21, 188 kb (rs17782313), and 109 kb (rs17700633) downstream of *MC4R* (13). Case-control studies confirmed associations of rs17782313 and rs17700633 with obesity, and a separate analysis identified a relationship between rs17782313 and morbid obesity (13). Low pairwise linkage disequilibrium was found between the two variants ($r^2 = 0.10$ in CEU HapMap). An independent genome-wide association study performed in Indian people identified four variants (rs12970134, rs477181, rs502933, and rs4450508) in high linkage disequilibrium (0.57–1.0 in CEU HapMap) ~150 kb downstream of *MC4R* associated with increased waist circumference, body weight, waist-to-hip ratio, and insulin resistance, of which the most strongly associated variant (rs12970134) (14) was in high linkage disequilibrium with rs17782313 (0.81 in CEU HapMap).

The aim of the present study was to examine the influence of rs17782313, rs17700633, rs12970134, rs477181, rs502933, and rs4450508 near *MC4R* on obesity-related quantitative traits in the general population of middle-aged people and to validate previously published associations of the variants with obesity (13,14) in the Danish popula-

From the [1]Steno Diabetes Center, Gentofte, Denmark; the [2]Institute of Medical Biochemistry and Genetics, University of Copenhagen, Copenhagen, Denmark; the [3]Institute of Preventive Medicine, Copenhagen University Hospital, Center for Health and Society, Copenhagen, Denmark; the [4]Department of General Practice, University of Aarhus, Aarhus, Denmark; the [5]Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; the [6]Faculty of Health Science, University of Aarhus, Aarhus, Denmark; the [7]Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark; and the [8]Faculty of Health Science, University of Southern Denmark, Odense, Denmark.

Corresponding author: Dorit Packert Zobel, dpaj@steno.dk.

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TABLE 1
Unadjusted anthropometric and metabolic characteristics of 5,807 treatment-naïve unrelated individuals from the population-based Inter99 sample stratified according to genotype of rs17782313, rs17700633, rs12970134, rs502933, or rs4450508 near *MC4R*

	Genotype			*P*	Effect per allele
rs17782313	T/T	T/C	C/C		
n (men/women)	3,291 (1,657/1,634)	2,159 (1,059/1,100)	357 (165/192)		
Age (years)	46 ± 8	46 ± 8	46 ± 8		
BMI (kg/m^2)	26.1 ± 4.5	26.4 ± 4.5	26.4 ± 4.7	0.01*	0.25 (0.06–0.43)
Weight (kg)	77.6 ± 15.9	78.7 ± 15.9	78.7 ± 17.1	6×10^{-4}*	1.04 (0.45–1.64)
Waist (cm)	86 ± 13	87 ± 13	87 ± 14	0.006*	0.67 (0.19–1.15)
Waist-to-hip ratio	0.85 ± 0.09	0.86 ± 0.09	0.85 ± 0.09	0.20	
Height (cm)	172.2 ± 9.3	172.4 ± 9.1	172.2 ± 8.9	0.02*	0.33 (0.06–0.61)
Fasting					
Plasma glucose (mmol/l)	5.5 ± 0.8	5.6 ± 0.8	5.5 ± 0.6	0.82	
Serum insulin (pmol/l)	42 ± 28	43 ± 28	39 ± 25	0.50	
Serum C-peptide (pmol/l)	591 ± 269	608 ± 282	576 ± 257	0.80	
Serum triglycerides (mmol/l)	1.3 ± 1.6	1.3 ± 1.1	1.3 ± 0.8	0.80	
Serum total cholesterol (mmol/l)	5.5 ± 1.1	5.5 ± 1.1	5.5 ± 1.0	0.23	
Serum HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	0.42	
HOMA-IR (pmol/l × mmol/l)	10.5 ± 8.2	10.8 ± 7.9	9.9 ± 6.7	0.53	
rs17700633	G/G	G/A	A/A		
n (men/women)	2,753 (1,376/1,377)	2,520 (1,255/1,265)	525 (253/272)		
Age (years)	46 ± 8	46 ± 8	46 ± 8		
BMI (kg/m^2)	26.1 ± 4.6	26.3 ± 4.5	26.6 ± 4.5	0.01*	0.23 (0.05–0.41)
Weight (kg)	77.8 ± 16.2	78.2 ± 15.7	79.3 ± 15.9	0.01*	0.71 (0.15–1.28)
Waist (cm)	86 ± 13	87 ± 13	87 ± 14	0.02*	0.53 (0.07–0.98)
Waist-to-hip ratio	0.85 ± 0.09	0.86 ± 0.09	0.86 ± 0.09	0.10	
Height (cm)	172.3 ± 9.4	172.3 ± 9.1	172.5 ± 9.1	0.43	
Fasting					
Plasma glucose (mmol/l)	5.5 ± 0.8	5.5 ± 0.7	5.6 ± 0.9	0.78	
Serum insulin (pmol/l)	42 ± 28	41 ± 28	43 ± 29	0.31	
Serum C-peptide (pmol/l)	593 ± 277	596 ± 269	610 ± 272	0.90	
Serum triglycerides (mmol/l)	1.3 ± 1.6	1.3 ± 1.1	1.4 ± 1.3	0.37	
Serum total cholesterol (mmol/l)	5.5 ± 1.1	5.6 ± 1.1	5.5 ± 1.1	0.81	
Serum HDL cholesterol (mmol/l)	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.06	
HOMA-IR (pmol/l × mmol/l)	10.7 ± 8.1	10.5 ± 8.0	10.9 ± 7.9	0.33	
rs12970134	G/G	G/A	A/A		
n (men/women)	2,982 (1,495/1,487)	2,339 (1,159/1,180)	465 (221/244)		
Age (years)	46 ± 8	46 ± 8	46 ± 8		
BMI (kg/m^2)	26.0 ± 4.5	26.4 ± 4.6	26.5 ± 4.6	7×10^{-4}*	0.31 (0.13–0.49)
Weight (kg)	77.4 ± 15.9	78.8 ± 16.0	79.0 ± 16.9	8×10^{-5}*	1.16 (0.58–1.73)
Waist (cm)	86 ± 13	87 ± 13	87 ± 14	3×10^{-4}*	0.85 (0.39–1.31)
Waist-to-hip ratio	0.85 ± 0.09	0.86 ± 0.09	0.86 ± 0.09	0.01*	0.003 (0.001–0.006)
Height (cm)	172.2 ± 9.3	172.4 ± 9.2	172.3 ± 8.8	0.06	
Fasting					
Plasma glucose (mmol/l)	5.5 ± 0.8	5.6 ± 0.8	5.5 ± 0.6	0.75	
Serum insulin (pmol/l)	42 ± 28	43 ± 28	41 ± 26	0.48	
Serum C-peptide (pmol/l)	588 ± 266	609 ± 287	581 ± 252	0.81	
Serum triglycerides (mmol/l)	1.3 ± 1.6	1.3 ± 1.1	1.3 ± 0.8	0.99	
Serum total cholesterol (mmol/l)	5.6 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.11	
Serum HDL cholesterol (mmol/l)	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.07	
HOMA-IR (pmol/l × mmol/l)	10.5 ± 8.0	10.8 ± 8.2	10.1 ± 7.2	0.45	
rs502933	C/C	C/A	A/A		
n (men/women)	2,416 (1,233/1,183)	2,582 (1,273/1,309)	744 (346/398)		
Age (years)	46 ± 8	46 ± 8	46 ± 8		
BMI (kg/m^2)	26.1 ± 4.5	26.3 ± 4.6	26.2 ± 4.4	0.16	
Weight (kg)	77.8 ± 15.9	78.4 ± 16.0	78.0 ± 16.3	0.03	
Waist (cm)	86 ± 13	87 ± 13	86 ± 13	0.06	
Waist-to-hip ratio	0.86 ± 0.09	0.86 ± 0.09	0.86 ± 0.09	0.10	
Height (cm)	172.3 ± 9.3	172.3 ± 9.1	172.3 ± 9.0	0.05	
Fasting					
Plasma glucose (mmol/l)	5.6 ± 0.9	5.6 ± 0.8	5.5 ± 0.7	0.69	
Serum insulin (pmol/l)	42 ± 28	43 ± 29	40 ± 25	0.47	
Serum C-peptide (pmol/l)	592 ± 272	603 ± 279	585 ± 252	0.85	
Serum triglycerides (mmol/l)	1.4 ± 1.7	1.3 ± 1.1	1.3 ± 0.9	0.19	
Serum total cholesterol (mmol/l)	5.6 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.09	
Serum HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.50	
HOMA-IR (pmol/l × mmol/l)	10.6 ± 8.2	10.7 ± 8.1	10.0 ± 7.0	0.43	

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TABLE 1
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	Genotype			*P*	Effect per allele
rs4450508	G/G	G/A	A/A		
n (men/women)	2,324 (1,191/1,133)	2,590 (1,286/1,304)	815 (383/432)		
Age (years)	46 ± 8	46 ± 8	46 ± 8		
BMI (kg/m^2)	26.1 ± 4.5	26.3 ± 4.6	26.1 ± 4.4	0.53	
Weight (kg)	77.9 ± 15.8	78.4 ± 16.0	77.8 ± 16.2	0.13	
Waist (cm)	87 ± 13	87 ± 13	86 ± 13	0.21	
Waist-to-hip ratio	0.86 ± 0.09	0.86 ± 0.09	0.86 ± 0.09	0.17	
Height (cm)	172.3 ± 9.4	172.4 ± 9.1	172.3 ± 9.1	0.04	
Fasting					
Plasma glucose (mmol/l)	5.5 ± 0.8	5.5 ± 0.8	5.5 ± 0.8	0.87	
Serum insulin (pmol/l)	42 ± 28	43 ± 29	40 ± 25	0.88	
Serum C-peptide (pmol/l)	591 ± 270	602 ± 280	588 ± 258	0.43	
Serum triglycerides (mmol/l)	1.4 ± 1.8	1.3 ± 1.0	1.3 ± 1.0	0.34	
Serum total cholesterol (mmol/l)	5.6 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.08	
Serum HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.40	
HOMA-IR (pmol/l × mmol/l)	10.6 ± 8.1	10.7 ± 8.2	10.1 ± 7.0	0.91	

Data are means ± SD (unadjusted) or effect size estimate (95% CI) unless otherwise indicated. Calculated *P* values and effect size estimates were adjusted for age and sex for obesity-related quantitative traits and height and for age, sex, and BMI for plasma glucose, serum insulin, serum C-peptide, serum lipids, and HOMA-IR. Values of plasma glucose, serum insulin, serum C-peptide, serum triglycerides, and HOMA-IR were logarithmically transformed before statistical analysis. HOMA-IR was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) divided by 22.5. *P* values were calculated assuming an additive model. **P* values remained significant after Benjamini-Hochberg correction.

tion. Finally, a potential association of these obesity-associated variants with type 2 diabetes was explored.

RESEARCH DESIGN AND METHODS

Case-control studies of obesity were performed in 14,940 Danish individuals from three study groups: *1*) a subgroup of the population-based Inter99 sample (*n* = 5,807) recruited from the Research Centre for Prevention and Health (ClinicalTrials.gov identifier: NCT00289237; ref. 15); *2*) the Danish subsample of the ADDITION (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen-Detected Diabetes in Primary Care) screening cohort (*n* = 8,487) sampled by the Department of General Practice at the University of Aarhus, Aarhus, Denmark (ClinicalTrials.gov identifier: NCT00237548; ref. 16); and *3*) a population-based group of unrelated middle-aged individuals (*n* = 646) examined at Steno Diabetes Center (SDC). Individuals with a previous diagnosis of type 2 diabetes were excluded from studies of obesity because medication might have affected body weight. Individuals were defined as lean (BMI <25 kg/m^2) or overweight/obese (≥25 kg/m^2). Furthermore, analyses were carried out on subgroups of obese (≥30 kg/m^2) or morbidly obese individuals (≥40 kg/m^2). Individuals were further stratified according to sex-specific waist circumference: in the latter context, we defined individuals with a waist circumference <80 cm (women) or <94 cm (men) as lean, overweight/abdominally obese individuals were ≥80 cm (women) or ≥94 cm (men), and a subgroup of abdominally obese individuals was characterized by waist circumference ≥88 cm (women) or ≥102 cm (men). Conditional analyses of case-control studies on BMI were performed in the Inter99, ADDITION, and the SDC study samples.

Case-control studies of type 2 diabetes (diagnosed according to the 1999 World Health Organization criteria [17]) were performed in 4,918 glucose-tolerant individuals and 3,903 case individuals from the above-mentioned cohorts plus a sample of unrelated type 2 diabetic patients from the outpatient clinic at SDC (*n* = 1,964). Further details of the study populations are found in Supplementary Tables A–B (available online at http://diabetes.diabetesjournals.org/cgi/content/full/db08-0620/DC1).

Studies of obesity-related quantitative traits, physical activity, and additive effects between rs12970134 near *MC4R* and *FTO* rs9939609 and conditional analyses on BMI as a quantitative trait were performed in the Inter99 cohort (*n* = 5,807), excluding individuals with a previous diagnosis of type 2 diabetes.

All participants were of self-reported Danish nationality, and informed written consent was obtained before participation. Studies were approved by regional ethics committees and were in accordance with the principles of the Helsinki Declaration II.

Anthropometrical and biochemical measurements. Body weight and height were measured with individuals wearing light clothing. Waist circumference was measured midway between the iliac crest and the lower costal margin. Blood samples were taken after an overnight fast of >8 h. Plasma glucose, serum insulin, C-peptide, and lipids were measured using SDC standard methods. The level of physical activity was self-reported by questionnaire (18).

Genotyping. Genotyping of rs17782313, rs17700633, rs12970134, rs477181, rs502933, and rs4450508 was performed using TaqMan allelic discrimination (KBioscience, Herts, U.K.). The genotyping success rates were >96%, and among 721 replicate samples error rates of 0.0%, 0.3%, 0.0%, 0.1%, 0.2%, and 0.1%, respectively, were observed. Genotype distributions obeyed Hardy-Weinberg equilibrium ($P > 0.05$).

Statistical analyses. For case-control studies, differences in genotype distributions were calculated applying an additive logistic regression model adjusted for sex and age. A general linear model tested variables of quantitative traits for differences between genotype groups and was adjusted for sex and age (and BMI where appropriate). For obesity-related analyses, the Benjamini-Hochberg method was used separately for each variant to correct for multiple testing (19). A test for homogeneity between the SDC population-based sample, Inter99, and the ADDITION cohort was performed for all variants by means of the Mantel-Haenszel method (fixed-effects model), and it revealed no heterogeneity between the studies ($P = 0.64–0.97$). Linear models were used to test for interaction between genotypes and physical activity on BMI or waist circumference using an ANOVA test. To assess whether variants affect body composition independently of each other, individual SNP contributions were analyzed by conditional analysis comparing linear models including single or pairwise genetic parameters. The combined effect of rs12970134 and *FTO* rs9939609 on BMI was assessed assuming additive effects of each variant ($P = 0.11$; equal effect sizes on BMI could be assumed for the two variants). Additivity was assessed comparing a linear model that assumed equal effects between rs12970134 and rs9939609 by summarizing the number of risk alleles as the genetic parameter with a model without a genetic parameter. Analyses were performed with RGui (version 2.5.0; R Development for Statistical Computing, Vienna). A *P* value <0.05 was considered significant.

RESULTS

Six variants near *MC4R* (rs17782313, rs17700633, rs12970134, rs502933, rs477181, and rs4450508) were genotyped in the Danish population, of which rs502933 and rs477181 were in complete linkage disequilibrium and, therefore, the results for rs477181 are not shown (pairwise linkage disequilibrium structure for the six variants are found in Supplementary Fig. 1). In the population-based Inter99 sample involving 5,807 treatment-naïve, unrelated individuals, rs17782313, rs17700633, and rs12970134 were associ-

TABLE 2
Genotype distributions, minor allele frequencies, and ORs for rs17782313, rs17700633, rs12970134, rs502933, and rs4450508 near *MC4R* among 14,940 individuals stratified according to genotype and BMI subgroup or sex-specific waist circumference subgroup

	n (men/women)	Genotype			Minor allele frequency	Additive model OR	*P*
rs17782313		T/T	T/C	C/C			
BMI							
<25 kg/m^2	4,721 (1,946/2,775)	2,721 (57)	1,722 (37)	278 (6)	24.1 (23.3–25.0)		
≥25 kg/m^2	10,219 (5,887/4,332)	5,651 (56)	3,915 (38)	653 (6)	25.5 (24.9–26.1)	1.09 (1.02–1.15)	0.006*
≥30 kg/m^2	3,913 (1,988/1,925)	2,136 (55)	1,507 (38)	270 (7)	26.2 (25.2–27.1)	1.12 (1.04–1.20)	0.003*
≥40 kg/m^2	283 (89/194)	148 (52)	112 (40)	23 (8)	27.9 (24.3–31.8)	1.20 (0.99–1.45)	0.06
Waist							
<80 (women) or <94 cm (men)	5,374 (2,834/2,540)	3,107 (58)	1,954 (36)	313 (6)	24.0 (23.2–24.8)		
≥80 (women) or ≥94 cm (men)	9,546 (4,986/4,560)	5,247 (55)	3,681 (38)	618 (7)	25.8 (25.1–26.4)	1.10 (1.03–1.16)	0.002*
≥88 (women) or ≥102 cm (men)	5,748 (2,720/3,028)	3,158 (55)	2,213 (38)	377 (7)	25.8 (25.0–26.6)	1.09 (1.02–1.16)	0.009*
rs17700633		G/G	G/A	A/A			
BMI							
<25 kg/m^2	4,697 (1,939/2,758)	2,296 (49)	2,002 (42)	399 (9)	29.8 (28.9–30.7)		
≥25 kg/m^2	10,135 (5,842/4,293)	4,685 (46)	4,427 (44)	1,023 (10)	31.9 (31.3–32.6)	1.12 (1.06–1.18)	8×10^{-5}*
≥30 kg/m^2	3,871 (1,964/1,907)	1,744 (45)	1,707 (44)	420 (11)	32.9 (31.9–34.0)	1.16 (1.08–1.24)	2×10^{-5}*
≥40 kg/m^2	278 (87/191)	108 (39)	143 (51)	27 (10)	35.4 (31.5–39.6)	1.30 (1.08–1.56)	0.005*
Waist							
<80 (women) or <94 cm (men)	5,346 (2,824/2,522)	2,575 (48)	2,319 (43)	452 (9)	30.1 (29.3–31.0)		
≥80 (women) or ≥94 cm (men)	9,467 (4,945/4,522)	4,394 (47)	4,104 (43)	969 (10)	31.9 (31.2–32.6)	1.09 (1.03–1.15)	0.002*
≥88 (women) or ≥102 cm (men)	5,684 (2,694/2,990)	2,598 (46)	2,495 (44)	591 (10)	32.3 (31.5–33.2)	1.10 (1.04–1.17)	0.001*
rs12970134		G/G	G/A	A/A			
BMI							
<25 kg/m^2	4,694 (1,934/2,760)	2,511 (54)	1,848 (39)	335 (7)	26.8 (25.9–27.7)		
≥25 kg/m^2	10,174 (5,869/4,305)	5,073 (50)	4,276 (42)	825 (8)	29.1 (28.5–29.8)	1.13 (1.07–1.20)	2×10^{-5}*
≥30 kg/m^2	3,886 (1,979/1,907)	1,922 (49)	1,631 (42)	333 (9)	29.6 (28.5–30.6)	1.15 (1.08–1.23)	6×10^{-5}*
≥40 kg/m^2	282 (88/194)	130 (46)	126 (45)	26 (9)	31.6 (27.7–35.6)	1.25 (1.04–1.50)	0.02*
Waist							
<80 (women) or <94 cm (men)	5,333 (2,814/2,519)	2,843 (53)	2,104 (40)	386 (7)	27.0 (26.1–27.8)		
≥80 (women) or ≥94 cm (men)	9,515 (4,976/4,539)	4,726 (50)	4,016 (42)	773 (8)	29.2 (28.6–29.9)	1.12 (1.06–1.19)	3×10^{-5}*
≥88 (women) or ≥102 cm (men)	5,710 (2,715/2,995)	2,831 (50)	2,412 (42)	467 (8)	29.3 (28.5–30.1)	1.12 (1.05–1.19)	4×10^{-4}*
rs502933		C/C	C/A	A/A			
BMI							
<25 kg/m^2	4,637 (1,904/2,733)	2,002 (43)	2,067 (45)	568 (12)	34.5 (33.6–35.5)		
≥25 kg/m^2	10,075 (5,801/4,274)	4,116 (41)	4,678 (46)	1,281 (13)	35.9 (35.3–36.6)	1.07 (1.02–1.13)	0.01
≥30 kg/m^2	3,851 (1,963/1,888)	1,562 (41)	1,786 (46)	503 (13)	36.3 (35.2–37.3)	1.08 (1.02–1.16)	0.01
≥40 kg/m^2	281 (91/190)	104 (37)	136 (48)	41 (15)	38.8 (34.7–43.0)	1.20 (1.01–1.43)	0.04
Waist							
<80 (women) or <94 cm (men)	5,275 (2,783/2,492)	2,284 (43)	2,346 (45)	645 (12)	34.5 (33.6–35.4)		
≥80 (women) or ≥94 cm (men)	9,418 (4,909/4,509)	3,824 (41)	4,393 (47)	1,201 (13)	36.1 (35.4–36.8)	1.08 (1.02–1.13)	0.006*
≥88 (women) or ≥102 cm (men)	5,662 (2,688/2,974)	2,281 (40)	2,666 (47)	715 (13)	36.2 (35.3–37.1)	1.07 (1.01–1.13)	0.02
rs4450508		G/G	G/A	A/A			
BMI							
< 25 kg/m^2	4,644 (1,916/2,728)	1,912 (41)	2,100 (45)	632 (14)	36.2 (35.2–37.2)		
≥25 kg/m^2	10,145 (5,855/4,290)	3,988 (39)	4,781 (47)	1,376 (14)	37.1 (36.5–37.8)	1.05 (0.99–1.10)	0.08
≥30 kg/m^2	3,873 (1,971/1,902)	1,510 (39)	1,820 (47)	543 (14)	37.5 (36.4–38.6)	1.06 (1.00–1.13)	0.06
≥40 kg/m^2	282 (86/196)	100 (36)	140 (49)	42 (15)	39.7 (35.7–43.9)	1.16 (0.98–1.38)	0.09

Continued on following page

ated with obesity-related quantitative traits of BMI (effect per minor allele 0.25 kg/m^2 [95% CI 0.06–0.43], 0.23 [0.05–0.41], and 0.31 [0.13–0.49], respectively; $P = 0.01$, 0.01, and 7×10^{-4}, respectively) and waist circumference (effect per minor allele 0.67 cm [0.19–1.15], 0.53 [0.07–0.98], and 0.85 [0.39–1.31]; $P = 0.006$, 0.02, and 3×10^{-4};

TABLE 2
Continued

	n (men/women)	Genotype			Minor allele frequency	Additive model OR	*P*
Waist							
<80 (women) or <94 cm (men)	5,293 (2,798/2,495)	2,192 (41)	2,391 (45)	710 (13)	36.0 (35.1–36.9)		
≥80 (women) or ≥94 cm (men)	9,477 (4,961/4,516)	3,700 (39)	4,484 (47)	1,239 (14)	37.3 (36.6–38.0)	1.06 (1.01–1.12)	0.03
≥88 (women) or ≥102 cm (men)	5,692 (2,701/2,991)	2,209 (39)	2,709 (47)	774 (14)	37.4 (36.5–38.3)	1.05 (1.00–1.12)	0.07

Data are *n* (%), minor allele frequency (95% CI), and OR (95% CI). Differences in genotype distribution between BMI classifications or waist circumference classifications were calculated applying an additive logistic regression model adjusted for sex and age. *P values remained significant after Benjamini-Hochberg correction.

although adjustment for BMI abolished these associations [data not shown]), and body weight (effect per minor allele 1.04 kg [0.45–1.64], 0.71 [0.15–1.28], and 1.16 [0.58–1.73]; $P = 6 \times 10^{-4}$, 0.01, and 8×10^{-5}) (Table 1).

rs17782313 was further positively associated with height (effect per minor allele 0.33 cm [0.06–0.61]; $P = 0.02$), whereas only rs12970134 was associated with waist-to-hip ratio (effect per minor allele 0.003 [0.001–0.006]; $P = 0.01$). No associations with fasting circulating levels of glucose, insulin, C-peptide, or lipids or with insulin resistance (homeostasis model assessment of insulin resistance [HOMA-IR]) were found for any of the six studied variants regardless of correction for BMI.

In case-control studies of 14,940 Danes from the Inter99, ADDITION, and the SDC study, samples dichotomized according to BMI or waist circumference. rs17782313, rs17700633, and rs12970134 were associated with obesity (Table 2; separate analyses in the three study samples are found in Supplementary Tables C–L). The minor C-allele of rs17782313 was associated with BMI-defined overweight/obesity and obesity (odds ratio [OR] 1.09 [95% CI 1.02–1.15], $P = 0.006$, and 1.12 [1.04–1.20], $P = 0.003$, respectively); however, the risk allele was only borderline associated with morbid obesity (1.20 [0.99–1.45]; $P = 0.06$). Also, the minor A-allele of rs17700633 was associated with overweight/obesity, obesity, and morbid obesity (1.12 [1.06–1.18], $P = 8 \times 10^{-5}$; 1.16 [1.08–1.24], $P = 2 \times 10^{-5}$; and 1.30 [1.08–1.56], $P = 0.005$). Likewise, the minor A-allele of rs12970134 was associated with overweight/obesity, obesity, and morbid obesity (1.13 [1.07–1.20], $P = 2 \times 10^{-5}$; 1.15 [1.08–1.23], $P = 6 \times 10^{-5}$; and 1.25 [1.04–1.50], $P = 0.02$). The frequencies of the potential minor risk alleles of rs502933, rs477181, and rs4450508 were higher among overweight/obese and obese subjects than among lean subjects; however, these differences did not reach statistical significance (Table 2; data not shown).

Moreover, the C-allele of rs17782313 was associated with overweight/abdominal obesity and abdominal obesity defined by sex-specific waist circumference (1.10 [1.03–1.16], $P = 0.002$, and 1.09 [1.02–1.16], $P = 0.009$, respectively). Likewise, the A-allele of rs17700633 was associated with overweight/abdominal obesity and abdominal obesity (1.09 [1.03–1.15], $P = 0.002$, and 1.10 [1.04–1.17], $P = 0.001$). Also, the A-allele of rs12970134 was associated with overweight/abdominal obesity and abdominal obesity (1.12 [1.06–1.19], $P = 3 \times 10^{-5}$, and 1.12 [1.05–1.19], $P = 4 \times 10^{-4}$). Finally, the minor A-allele of rs502933 was associated with waist circumference–defined overweight/abdominal obesity (1.08 [1.02–1.13]; $P = 0.006$).

Conditional analyses were performed to differentiate between the effects on BMI of rs12970134, rs17782313, and rs17700633. In studies of BMI as a quantitative trait, we found that the association of rs12970134 remained significant after analyses conditional on rs17782313 and rs17700633, respectively. In contrast, all effects on BMI of rs17782313 and rs17700633 were abolished when taking the effect of rs12970134 into account (data not shown). However, in case-control studies of BMI, both rs12970134 and rs17700633 had independent effects (data not shown).

In case-control studies including 4,918 glucose-tolerant individuals and 3,903 patients with type 2 diabetes, the frequencies of the minor risk alleles of rs17782313 and rs12970134 were borderline associated with type 2 diabetes (OR 1.08 [0.99–1.18], $P = 0.08$, and 1.08 [1.00–1.18], $P = 0.06$, respectively) (Table 3); however, these borderline associations were abolished after adjustment for BMI (1.04 [0.94–1.14], $P = 0.48$, and 1.03 [0.93–1.13], $P = 0.57$). For rs17700633, rs502933, rs477181, rs502933, and rs4450508, the frequencies of the minor risk alleles were also higher among patients with type 2 diabetes; however, these differences did not reach statistical significance (Table 3; data not shown).

A potential interaction between the six variants' genotypes (analyzed separately) and self-reported physical activity on BMI or waist circumference was investigated in the population-based Inter99 cohort. We found no impact of genotype on BMI or waist circumference associated with the level of physical activity (data not shown).

In the population-based Inter99 sample of adults, an additive effect between rs12970134 near *MC4R* and *FTO* rs9939609 on BMI was found (Fig. 1*A* and *B*). The impact on BMI was 0.43 kg/m^2 (95% CI 0.30–0.55) per risk allele ($P = 1 \times 10^{-11}$ for additivity). Comparisons of linear models showed that the *FTO* rs9939609 has the largest effect; however, rs12970134 near *MC4R* contributed to this association on top of rs9939609 (Fig. 1*A*).

DISCUSSION

Population-based studies of obesity-related quantitative traits in the Danish population confirmed previously identified associations of rs17782313, rs17700633, and rs12970134 near *MC4R* with obesity (13,14). The effect sizes per minor risk allele on BMI for rs17782313 (0.25

TABLE 3
Genotype distributions, minor allele frequencies, and ORs for rs17782313, rs17700633, rs12970134, rs502933, and rs4450508 near *MC4R* among 8,821 individuals with normal glucose tolerance and patients with type 2 diabetes

	n (men/women)	Genotype			Minor allele frequency (95% CI)	Additive model [OR (95% CI)]*	*P*	Additive model [OR (95% CI)]†	*P*
rs17782313		T/T	T/C	C/C					
Normal glucose tolerance	4,918 (2,276/2,642)	2,802 (57)	1,810 (37)	306 (6)	24.6 (23.8–25.5)				
Type 2 diabetes	3,903 (2,317/1,586)	2,135 (55)	1,500 (38)	268 (7)	26.1 (25.1–27.1)	1.08 (0.99–1.18)	0.08	1.04 (0.94–1.14)	0.48
rs17700633		G/G	G/A	A/A					
Normal glucose tolerance	4,911 (2,283/2,628)	2,349 (48)	2,114 (43)	448 (9)	30.6 (29.7–31.6)				
Type 2 diabetes	3,881 (2,305/1,576)	1,841 (47)	1,658 (43)	382 (10)	31.2 (30.2–32.2)	1.05 (0.97–1.14)	0.26	1.00 (0.91–1.09)	0.96
rs12970134		G/G	G/A	A/A					
Normal glucose tolerance	4,882 (2,261/2,621)	2,544 (52)	1,945 (40)	393 (8)	28.0 (27.1–28.9)				
Type 2 diabetes	3,839 (2,310/1,580)	1,942 (50)	1,631 (42)	320 (8)	29.2 (28.2–30.2)	1.08 (1.00–1.18)	0.06	1.03 (0.93–1.13)	0.57
rs502933		C/C	C/A	A/A					
Normal glucose tolerance	4,843 (2,244/2,599)	2,041 (42)	2,165 (45)	657 (13)	35.5 (34.6–36.5)				
Type 2 diabetes	3,834 (2,270/1,561)	1,518 (40)	1,822 (47)	494 (13)	36.6 (35.6–37.7)	1.07 (0.98–1.16)	0.11	1.04 (0.95–1.14)	0.34
rs4450508		G/G	G/A	A/A					
Normal glucose tolerance	4,845 (2,253/2,592)	1,975 (41)	2,183 (45)	687 (14)	36.7 (35.7–37.7)				
Type 2 diabetes	3,860 (2,289/1,568)	1,460 (38)	1,857 (48)	543 (14)	38.1 (37.0–39.2)	1.07 (0.99–1.16)	0.08	1.07 (0.98–1.16)	0.16

Data are *n* (%), minor allele frequency (95% CI), and OR (95% CI). *P* values compare differences in genotype distribution between normally glucose-tolerant individuals and patients with type 2 diabetes applying an additive logistic regression model adjusted for sex and age (*) or sex, age and BMI (†).

kg/m^2) and rs17700633 (0.23 kg/m^2) were slightly larger than the previous findings (0.22 and 0.15 kg/m^2, respectively [13]), whereas individuals homozygous for the minor allele of rs12970134 had a ~1.7 cm increased waist circumference, which was slightly lower than the previous findings (~2 cm [ref. 14]). ORs for overweight/obesity (1.09) and obesity (1.12) defined by BMI for rs17782313 (Table 2) were comparable with previous findings (1.08 and 1.12, respectively) (13), whereas the effect on morbid obesity (1.20) was less pronounced in the Danish population than in the previously reported morbid obesity case-control study (1.31) (13). This association was only borderline significant in the Danish population; however, this was probably due to a limited sample size ($n_{\text{morbid-obese}}$ = 283), and the less pronounced effect in the Danish population is likely due to a lower mean BMI in the Danish morbidly obese case individuals. Naturally, rs12970134 was also associated with overweight, obesity, and morbid obesity in the Danish population, as this variant was in high linkage disequilibrium with rs17782313 (r^2 = 0.76 in the Danish Inter99 population). A novel association between morbid obesity and the A-allele of rs17700633 was identified, suggesting a more pronounced effect on extreme obesity of this variant. Case-control studies of sex-specific waist circumference, a surrogate measure of abdominal obesity, showed ORs comparable with our studies of BMI-defined obesity.

In population-based studies of obesity-related quantitative traits, rs17782313, rs17700633, and rs12970134 were associated with waist circumference. However, adjustment for BMI abolished these associations, which may indicate an effect on global rather than abdominal obesity; this effect may be further supported by the fact that waist circumference, but not waist-to-hip ratio, was associated with these variants. Furthermore, in agreement with previous findings, rs17782313 was positively associated with height (13), pointing toward an influence on overall body size for this variant. A possible association of rs12970134, rs477181, rs502933, or rs4450508 with waist-to-hip ratio (except rs12970134) and insulin resistance (HOMA-IR) (14) was not replicated in the Danish population-based study sample; likewise, associations of rs477181, rs502933, and rs4450508 with BMI, waist circumference, or body weight were also not replicated. A difference in ethnicity may possibly explain the divergent results because only ~38% of the individuals in the publication by Chambers et al. (14) were of European ancestry, whereas ~62% were of Indian Asian ancestry.

Conditional analyses of BMI as a quantitative trait showed that the BMI effects of rs17782313 and rs17700633 were dependent on the BMI effect of rs12970134. A likely explanation is that rs12970134 is in higher linkage disequilibrium with the causal variant than rs17782313 and rs17700633. However, in conditional analyses of case-control studies of BMI, both rs12970134 and rs17700633 had independent effects. Therefore, we cannot exclude an independent effect of rs17700633 on obesity, and this suggests that rs17700633, located in a linkage disequilibrium block adjacent to rs12970134 and rs17782313, may represent a different variant associated with obesity.

Because obesity is a predisposing factor for type 2 diabetes, we investigated a possible relationship between the six variants near *MC4R* and type 2 diabetes. We identified borderline significant associations for the obesity-associated risk alleles of rs17782313 and rs12970134 with type 2 diabetes (OR 1.08 for both variants), whereas potential associations with type 2 diabetes did not reach

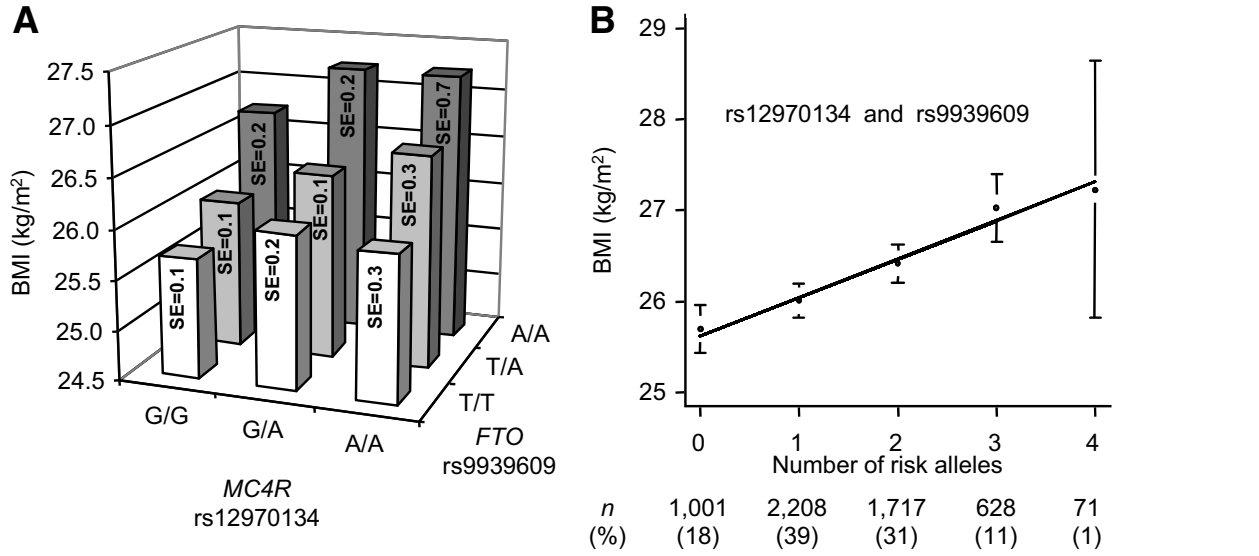

FIG. 1. Additive effect of rs12970134 near *MC4R* and *FTO* rs9939609 genotypes on BMI (n = 5,625). *A*: Values are means; SEs are shown on the bars. *B*: Values are means ± SE. Risk alleles are the minor A-alleles of rs12970134 and *FTO* rs9939609, respectively. The effect on BMI (adjusted for age and sex) was 0.43 kg/m^2 (95% CI 0.30–0.55 kg/m^2) per risk allele ($P = 1 \times 10^{-11}$ for additivity).

statistical significance for the other four variants near *MC4R*. The potential associations of rs17782313 and rs12970134 with type 2 diabetes were abolished when adjusting for BMI, indicating that a diabetogenic effect might be mediated through an increase in BMI, analogous to the association of variation in *FTO* with type 2 diabetes (10,20). The increased risk of type 2 diabetes via obesity is presumably through insulin resistance, although no difference in HOMA-IR (regardless of correction for BMI) was found between individuals with or without the risk alleles. We estimated statistical power to detect the expected effect of variants near *MC4R* on HOMA-IR based on the *MC4R* effect on BMI and the correlation between BMI and HOMA-IR, and we found a statistical power below 50% for all variants. Therefore, the lack of association of variants near *MC4R* with HOMA-IR may be due to low statistical power in the present study.

The present study demonstrated an additive effect between rs12970134 near *MC4R* and *FTO* rs9939609 on BMI (Fig. 1*A* and *B*). The *FTO* rs9939609 had the largest effect; however, rs12970134 near *MC4R* contributed to this association on top of rs9939609.

It is still unsettled which variants in the genomic region are causal. Loos et al. (13) showed that the associations with obesity were not secondary to an association between the *MC4R* Val103Ile polymorphism and lower risk of obesity (13). Given that rs17782313, rs12970134, and rs17700633 are located between 188 and 109 kb downstream of *MC4R*, it is also unknown whether the causal variants actually influence regulation of *MC4R*, although this is the most likely biological candidate gene in the region, as the pattern of phenotypic association is consistent with an effect mediated through altered MC4R function. Therefore, variants mapping far away from adjacent coding sequences may modulate phenotypes through remote effects on expression or translation.

In summary, we have extended the present knowledge of rs17782313, rs17700633, and rs12970134 near *MC4R* and validated that the variants associate with various measures of obesity in the Danish population. These variants may only be markers in linkage disequilibrium with the causal variants, and future studies identifying functional evidence linking rs17782313, rs17700633, and rs12970134 to expression of *MC4R* or another obesity-related gene are needed.

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REFERENCES

1. Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S: A frameshift mutation in *MC4R* associated with dominantly inherited human obesity. *Nat Genet* 20:111–112, 1998
2. Vaisse C, Clement K, Guy-Grand B, Froguel P: A frameshift mutation in human *MC4R* is associated with a dominant form of obesity. *Nat Genet* 20:113–114, 1998
3. Larsen LH, Echwald SM, Sørensen TI, Andersen T, Wulff BS, Pedersen O: 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, 2005
4. Gantz I, Miwa H, Konda Y, Shimoto Y, Tashiro T, Watson SJ, DelValle J,

Yamada T: Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. *J Biol Chem* 268:15174–15179, 1993
5. Mountjoy KG, Wild JM: Melanocortin-4 receptor mRNA expression in the developing autonomic and central nervous systems. *Brain Res Dev Brain Res* 107:309–314, 1998
6. Kask A, Rägo L, Wikberg JE, Schiöth HB: Evidence for involvement of the melanocortin MC4 receptor in the effects of leptin on food intake and body weight. *Eur J Pharmacol* 360:15–19, 1998
7. Young EH, Wareham NJ, Farooqi S, Hinney A, Hebebrand J, Scherag A, O'Rahilly S, Barroso I, Sandhu MS: The V103I polymorphism of the *MC4R* gene and obesity: population based studies and meta-analysis of 29,563 individuals. *Int J Obes (Lond)* 31:1437–1441, 2007
8. Stutzmann F, Vatin V, Cauchi S, Morandi A, Jouret B, Landt O, Tounian P, Levy-Marchal C, Buzzetti R, Pinelli L, Balkau B, Horber F, Bougnères P, Froguel P, Meyre D: Non-synonymous polymorphisms in melanocortin-4 receptor protect against obesity: the two facets of a Janus obesity gene. *Hum Mol Genet* 16:1837–1844, 2007
9. Heid IM, Vollmert C, Kronenberg F, Huth C, Ankerst DP, Luchner A, Hinney A, Brönner G, Wichmann HE, Illig T, Döring A, Hebebrand J: Association of the *MC4R* V103I polymorphism with the metabolic syndrome: the KORA Study. *Obesity* (*Silver Spring*) 16:369–376, 2008
10. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI: A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316:889–894, 2007
11. Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P: Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724–726, 2007
12. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orrú M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR: Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet* 3:e115, 2007
13. Loos RJF, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, Inouye M, Freathy RM, Attwood AP, Beckmann JS, Berndt SI, Bergmann S, Bennett AJ, Bingham SA, Bochud M, Brown M, Cauchi S, Connell JM, Cooper C, Smith GD, Day I, Dina C, De S, Dermitzakis ET, Doney ASF, Elliott KS, Elliott P, Evans DM, Sadaf Farooqi I, Froguel P, Ghori J, Groves CJ, Gwilliam R, Hadley D, Hall AS, Hattersley AT, Hebebrand J, Heid IM, Herrera B, Hinney A, Hunt SE, Jarvelin MR, Johnson T, Jolley JDM, Karpe F, Keniry A, Khaw KT, Luben RN, Mangino M, Marchini J, McArdle WL, McGinnis R, Meyre D, Munroe PB, Morris AD, Ness AR, Neville MJ, Nica AC, Ong KK, O'Rahilly S, Owen KR, Palmer CNA, Papadakis K, Potter S, Pouta A, Qi L, Randall JC, Rayner NW, Ring SM, Sandhu MS, Scherag A, Sims MA, Song K, Soranzo N, Speliotes EK, Syddall HE, Teichmann SA, Timpson NJ, Tobias JH, Uda M, Ganz Vogel CI, Wallace C, Waterworth DM, Weedon MN, Willer CJ, Wraight VL, Yuan X, Zeggini E, Hirschhorn JN, Strachan DP, Ouwehand WH, Caulfield MJ, Samani NJ, Frayling TM, Vollenweider P, Waeber G, Mooser V, Deloukas P, McCarthy MI, Wareham NJ, Barroso I: Common variants near *MC4R* are associated with fat mass, weight and risk of obesity. *Nat Genet* 40:768–775, 2008
14. Chambers JC, Elliott P, Zabaneh D, Zhang W, Li Y, Froguel P, Balding D, Scott J, Kooner JS: Common genetic variation near *MC4R* is associated with waist circumference and insulin resistance. *Nat Genet* 40:716–718, 2008
15. Jørgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glümer C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. *Eur J Cardiovasc Prev Rehabil* 10:377–386, 2003
16. Lauritzen T, Griffin S, Borch-Johnsen K, Wareham NJ, Wolffenbuttel BH, Rutten G, Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen Detected Diabetes in Primary Care: The ADDITION study: proposed trial of the cost-effectiveness of an intensive multifactorial intervention on morbidity and mortality among people with type 2 diabetes detected by screening. *Int J Obes Relat Metab Disord* 24 (Suppl. 3):S6–S11, 2000
17. World Health Organization: *Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus*. Geneva, World Health Org., 1999 (Tech. Rep. Ser., no. WHO/NCD/NCS/99.2)
18. Glümer C, Jørgensen T, Borch-Johnsen K: Prevalences of diabetes and impaired glucose regulation in a Danish population: the Inter99 study. *Diabetes Care* 26:2335–2340, 2003
19. Hochberg Y, Benjamini Y: More powerful procedures for multiple significance testing. *Stat Med* 9:811–818, 1990
20. Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, Nielsen AL, Albrechtsen A, Borch-Johnsen K, Rasmussen SS, Clausen JO, Sandbaek A, Lauritzen T, Hansen L, Jørgensen T, Pedersen O, Hansen T: Low physical activity accentuates the effect of the *FTO* rs9939609 polymorphism on body fat accumulation. *Diabetes* 57:95–101, 2008