Variants near \textit{MC4R} associate with obesity and influence obesity-related quantitative traits in a population of middle-aged people: studies of 14,940 Danes

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ABSTRACT

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, for association with obesity in 14,940 individuals, and for association with type 2 diabetes in 8,821 individuals.

Results: The minor risk alleles of rs17782313, rs17700633, and rs12970134 associated with BMI (effect per-allele: 0.25 [$P=0.01$], 0.23 [$P=0.01$], and 0.31 [$P=7\times10^{-4}$] kg/m$^2$, respectively); waist circumference (effect per-allele: 0.67 [$P=0.006$], 0.53 [$P=0.02$], and 0.85 [$P=3\times10^{-4}$] cm, respectively); and body weight (effect per-allele: 1.04 [$P=6\times10^{-4}$], 0.71 [$P=0.01$], and 1.16 [$P=8\times10^{-5}$] kg, respectively). In case-control studies of obesity defined by BMI the minor C-allele of rs17782313 associated with overweight/obesity and obesity (OR$_{overweight/obesity}=1.09$, $P=0.006$; OR$_{obesity}=1.12$, $P=0.003$). Similarly, the minor A-allele of rs17700633 associated with overweight/obesity and obesity (OR$_{overweight/obesity}=1.12$, $P=8\times10^{-5}$; OR$_{obesity}=1.16$, $P=2\times10^{-5}$), and the minor A-allele of rs12970134 was also associated with overweight/obesity and obesity (OR$_{overweight/obesity}=1.13$, $P=2\times10^{-5}$; OR$_{obesity}=1.15$, $P=6\times10^{-5}$). rs477181, rs502933, and rs4450508 were not statistically significantly associated with obesity in the Danish population. The frequency of the minor risk alleles of rs17782313 and rs12970134 were higher among patients with type 2 diabetes compared with glucose-tolerant individuals (OR=1.08, $P=0.08$; and OR=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.
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), 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 (GWA) data from white Europeans informative for body mass index (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 pair-wise linkage disequilibrium (LD) was found between the two variants ($r^2=0.10$ in CEU HapMap). An independent GWA study performed in Indian people identified four variants (rs12970134, rs477181, rs502933, and rs4450508), in high LD ($r^2$: 0.57-1.0 in CEU HapMap) approximately 150 kb downstream of MC4R associating 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 LD with rs17782313 ($r^2=0.81$ 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 population. Finally, a potential association of these obesity-associated variants with type 2 diabetes was explored.

**RESEARCH DESIGN AND METHODS**

*Subjects.* 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 Research Centre for Prevention and Health (ClinicalTrials.gov Identifier: NCT00289237, (15)); 2) The Danish sub-sample of 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, Denmark (ClinicalTrials.gov Identifier: NCT00237548, (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 as medication might affect body weight. Individuals were defined as lean (BMI $<25$ kg/m$^2$) or overweight/obese (BMI $\geq 25$ kg/m$^2$). Furthermore, analyses were carried out on subgroups of obese (BMI $\geq 30$ kg/m$^2$) or morbidly obese individuals (BMI $\geq 40$ 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)
Variants near \textit{MC4R} associate with obesity or <94 cm (men) as lean, whereas overweight/abdominally obese individuals were \( \geq 80 \text{ cm (women)} \) or \( \geq 94 \text{ cm (men)} \), and a subgroup of abdominally obese individuals were \( \geq 88 \text{ cm (women)} \) or \( \geq 102 \text{ cm (men)} \) around the waist. 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 WHO 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 diabetes patients from the outpatient clinic at SDC \( (n=1,964) \). Further details of the study populations are found in Supplementary Tables A-B.

Studies of obesity-related quantitative traits, physical activity, additive effects between rs12970134 near \textit{MC4R} and \textit{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.

\textit{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 \( >8 \text{ hours} \). Plasma glucose, serum insulin, C-peptide, and lipids were measured using Steno Diabetes Center standard methods. The level of physical activity was self-reported by questionnaire (18).

\textit{Genotyping.} Genotyping of rs17782313, rs17700633, rs12970134, rs477181, rs502933, and rs4450508 was performed using TaqMan allelic discrimination (KBioscience, Herts, UK). 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 \( (P>0.05) \).

\textit{Statistical analyses.} For case-control studies differences in genotype distributions were calculated applying an additive logistic regression model while adjusting for sex and age. A general linear model tested variables of quantitative traits for differences between genotype groups and were 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 revealed no heterogeneity between studies \( (P=0.64-0.97) \). Linear models were used to test for interaction between genotype and physical activity on BMI or waist circumference using an ANOVA test. To assess whether variants affect body composition independent from each other, individual SNP contribution was analyzed by conditional analysis independent from each other, individual SNP contribution was analyzed by conditional analysis comparing linear models including a single or pair-wise genetic parameter(s), respectively. The combined effect of rs12970134 and \textit{FTO} rs9939609 on BMI was assessed assuming additive effects of each variant (equal effect sizes on BMI could be assumed for the two variants \[ P=0.11 \]). Additivity was assessed comparing a linear model assuming 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 (http://www.r-project.org/). 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 LD, and therefore the results for rs477181 are not shown (pairwise LD structure for the six variants are found in Supplementary Figure 1). In the population-based Inter99 sample involving 5,807 treatment-naïve, unrelated individuals rs17782313, rs17700633, and rs12970134 were associated with obesity-related quantitative traits of BMI (effect per minor allele: 0.25 [95% CI 0.06-0.43], 0.23 [0.05-0.41], and 0.31 [0.13-0.49] kg/m², respectively; P=0.01, 0.01, and 7×10⁻⁴, respectively); waist circumference (effect per minor allele: 0.67 [0.19-1.15], 0.53 [0.07-0.98], and 0.85 [0.39-1.31] cm, respectively; P=0.006, 0.02, and 3×10⁻⁴, respectively), although adjustment for BMI abolished these associations (data not shown); and body weight (effect per minor allele: 1.04 [0.45-1.64], 0.71 [0.15-1.28], and 1.16 [0.58-1.73] kg, respectively; P=6×10⁻⁴, 0.01, and 8×10⁻⁵, respectively) (Table 1).

rs17782313 was further positively associated with height (effect per minor allele: 0.33 [0.06-0.61] cm, P=0.02) while 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, lipids, or with 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 overweight/obesity and obesity defined by BMI (Odds ratio [OR] overweight/obesity=1.09 [1.02-1.15], P=0.006; OR obesity=1.12 [1.04-1.20], P=0.003); however, the risk allele was only borderline associated with morbid obesity (OR 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 (OR overweight/obesity=1.12 [1.06-1.18], P=8×10⁻⁵; OR obesity=1.16 [1.08-1.24], P=2×10⁻⁵; and OR morbid-obesity=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 (OR overweight/obesity=1.13 [1.07-1.20], P=2×10⁻⁵; OR obesity=1.15 [1.08-1.23], P=6×10⁻⁵; and OR morbid-obesity=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 compared with lean subjects; however, these differences did not reach statistical significance (Table 2 and data not shown).

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

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 frequency 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 OR=1.08 [1.00-1.18], $P=0.06$, respectively) (Table 3); however, these borderline associations were abolished after adjustment for BMI (OR=1.04 [0.94-1.14], $P=0.48$; and OR=1.03 [0.93-1.13], $P=0.57$, respectively). 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 and 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 (Figure 1A and B). The impact on BMI was 0.43 (95% CI 0.30-0.55) kg/m$^2$ 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 (Figure 1A).

**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 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)), while individuals homozygous for the minor allele of rs12970134 had a $\sim$1.7 cm increased waist circumference, which was slightly lower than the previous findings ($\sim$2 cm, (14)). ORs for overweight/obesity ($\text{OR}_{\text{overweight/obesity}}=1.09$) and obesity ($\text{OR}_{\text{obesity}}=1.12$) defined by BMI for rs17782313 (Table 2) were comparable with previous findings ($\text{OR}_{\text{overweight/obesity}}=1.08$, $\text{OR}_{\text{obesity}}=1.12$ (13)), while the effect on morbid obesity ($\text{OR}_{\text{morbid-obesity}}=1.20$) was less pronounced in the Danish population compared with the previously reported morbid obesity case-control study ($\text{OR}_{\text{morbid-obesity}}=1.31$) (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 morbid obese case individuals. Naturally, rs12970134 was also associated with overweight, obesity and morbid obesity in the Danish population, as this variant was in high LD 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, as adjustment for BMI abolished these associations, this may indicate an effect on global rather than abdominal obesity, which further may be 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 towards 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, and neither were associations of rs477181, rs502933, or rs4450508 with BMI, waist circumference or body weight. A difference in ethnicity is a possibility to explain the divergent results, as 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 LD 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 can not exclude an independent effect of rs17700633 on obesity, and this suggests that rs17700633, located in an adjacent LD block to rs12970134 and rs17782313, may represent a different variant associated with obesity.

As 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 risks alleles of rs17782313 and rs12970134 with type 2 diabetes (OR_{type-2-diabetes}=1.08 for both variants), whereas potential associations with type 2 diabetes did not reach 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 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 (Figure 1A 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. In the report by Loos and co-workers it was shown that the
associations with obesity were not secondary to an association between the MC4R Val103Ile polymorphism and lower risk of obesity (7,13). Realizing 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. It therefore seems that variants mapping far away from adjacent coding sequence may modulate phenotypes apparently through remote effects on expression or translation.

In conclusion 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 LD 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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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 | T/T | T/C | C/C |
|----------|-----|-----|-----|
| n (m/w)  | 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/m²) | 26.1±4.5 | 26.4±4.5 | 26.4±4.7 |
| Weight (kg) | 77.6±15.9 | 78.7±15.9 | 78.7±17.1 |
| Waist (cm) | 86±13 | 87±13 | 87±14 |
| Waist-to-Hip ratio | 0.85±0.09 | 0.86±0.09 | 0.85±0.09 |
| Height (cm) | 172.2±9.3 | 172.4±9.1 | 172.2±8.9 |
| Fasting: | | | |
| p-glucose (mmol/l) | 5.5±0.8 | 5.6±0.8 | 5.5±0.6 |
| s-insulin (pmol/l) | 42±28 | 43±28 | 39±25 |
| s-C-peptide (pmol/l) | 591±269 | 608±282 | 576±257 |
| s-triglycerides (mmol/l) | 1.3±1.6 | 1.3±1.1 | 1.3±0.8 |
| s-total cholesterol (mmol/l) | 5.5±1.1 | 5.5±1.1 | 5.5±1.0 |
| s-HDL cholesterol (mmol/l) | 1.4±0.4 | 1.4±0.4 | 1.5±0.4 |
| HOMA-IR (pmol/l × mmol/l) | 10.5±8.2 | 10.8±7.9 | 9.9±6.7 |

| rs17700633 | G/G | G/A | A/A |
|------------|-----|-----|-----|
| n (m/w)    | 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/m²) | 26.1±4.6 | 26.3±4.5 | 26.6±4.5 |

* p < 0.05, CI = confidence interval.
Variants near *MC4R* associate with obesity

|                             | 77.8±16.2 | 78.2±15.7 | 79.3±15.9 | 0.01* | 0.71 (0.15-1.28) |
|-----------------------------|-----------|-----------|-----------|-------|-----------------|
| Weight (kg)                 | 86±13     | 87±13     | 87±14     | 0.02* | 0.53 (0.07-0.98) |
| Waist (cm)                  | 0.85±0.09 | 0.86±0.09 | 0.86±0.09 | 0.10  |                 |
| Waist-to-Hip ratio          | 172.3±9.4 | 172.3±9.1 | 172.5±9.1 | 0.43  |                 |
| Height (cm)                 |           |           |           |       |                 |
| Fasting:                    |           |           |           |       |                 |
| p-glucose (mmol/l)          | 5.5±0.8   | 5.5±0.7   | 5.6±0.9   | 0.78  |                 |
| s-insulin (pmol/l)          | 42±28     | 41±28     | 43±29     | 0.31  |                 |
| s-C-peptide (pmol/l)        | 593±277   | 596±269   | 610±272   | 0.90  |                 |
| s-triglycerides (mmol/l)    | 1.3±1.6   | 1.3±1.1   | 1.4±1.3   | 0.37  |                 |
| s-total cholesterol (mmol/l)| 5.5±1.1   | 5.6±1.1   | 5.5±1.1   | 0.81  |                 |
| s-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 (m/w)                     | 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/m²)                 | 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:                    |           |           |           |       |                 |
| p-glucose (mmol/l)          | 5.5±0.8   | 5.6±0.8   | 5.5±0.6   | 0.75  |                 |
| s-insulin (pmol/l)          | 42±28     | 43±28     | 41±26     | 0.48  |                 |
| s-C-peptide (pmol/l)        | 588±266   | 609±287   | 581±252   | 0.81  |                 |
| s-triglycerides (mmol/l)    | 1.3±1.6   | 1.3±1.1   | 1.3±0.8   | 0.99  |                 |
| s-total cholesterol (mmol/l)| 5.6±1.1   | 5.5±1.1   | 5.5±1.1   | 0.11  |                 |
| s-HDL cholesterol (mmol/l)  | 1.5±0.4   | 1.4±0.4   | 1.4±0.4   | 0.07  |                 |
Variants near *MC4R* associate with obesity

|                      | rs502933 |                      | rs4450508 |
|----------------------|----------|----------------------|-----------|
|                      | C/C      | C/A                  | G/G       |
| n (m/w)              | 2,416 (1,233/1,183) | 2,582 (1,273/1,309) | 2,324 (1,191/1,133) |
| Age (years)          | 46±8     | 46±8                 | 46±8      |
| BMI (kg/m²)          | 26.1±4.5 | 26.3±4.6             | 26.1±4.5  |
| Weight (kg)          | 77.8±15.9| 78.4±16.0            | 77.9±15.8 |
| Waist (cm)           | 86±13    | 87±13                | 87±13     |
| Waist-to-Hip ratio   | 0.86±0.09| 0.86±0.09            | 0.86±0.09 |
| Height (cm)          | 172.3±9.3| 172.3±9.1            | 172.3±9.4 |
| Fasting:             |          |                      |           |
| p-glucose (mmol/l)   | 5.6±0.9  | 5.6±0.8              | 5.6±1.1   |
| s-insulin (pmol/l)   | 42±28    | 43±29                | 42±28     |
| s-C-peptide (pmol/l) | 592±272  | 603±279              | 592±272   |
| s-triglycerides (mmol/l) | 1.4±1.7 | 1.3±1.1              | 1.4±0.4   |
| s-total cholesterol (mmol/l) | 5.6±1.1 | 5.5±1.1              | 5.6±1.1   |
| s-HDL cholesterol (mmol/l) | 1.4±0.4 | 1.4±0.4              | 1.4±0.4   |
| HOMA-IR (pmol/l × mmol/l) | 10.5±8.0 | 10.8±8.2             | 10.6±8.2  |

The table above presents the associations between variants near *MC4R* and obesity-related traits. Each variant (rs502933 and rs4450508) is compared with its respective genotype groups (C/C, C/A, A/A) for various anthropometric and metabolic measures. The table includes measurements such as BMI, weight, waist, fasting glucose, insulin, C-peptide, triglycerides, total cholesterol, HDL cholesterol, and HOMA-IR. The data show statistical differences across genotypes for each measure, indicating potential genetic influences on these traits.
Variants near *MC4R* associate with obesity

| Parameter                        | Group 1     | Group 2     | Group 3     | P-value |
|----------------------------------|-------------|-------------|-------------|---------|
| p-glucose (mmol/l)               | 5.5±0.8     | 5.5±0.8     | 5.5±0.8     | 0.87    |
| s-insulin (pmol/l)               | 42±28       | 43±29       | 40±25       | 0.88    |
| s-C-peptide (pmol/l)             | 591±270     | 602±280     | 588±258     | 0.43    |
| s-triglycerides (mmol/l)         | 1.4±1.8     | 1.3±1.0     | 1.3±1.0     | 0.34    |
| s-total cholesterol (mmol/l)     | 5.6±1.1     | 5.5±1.1     | 5.5±1.1     | 0.08    |
| s-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). 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. p, plasma; s, serum; HOMA-IR: homeostasis model assessment of insulin resistance index.
Table 2

Genotype distributions, minor allele frequencies and odds ratios 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.

| rs17782313 | n (m/w) | Genotype | MAF (95% CI) | Additive model OR (95% CI) |
|------------|---------|----------|--------------|---------------------------|
| BMI        |         |          |              |                           |
| < 25 kg/m² | 4,721 (1,946/2,775) | T/T      | 2,721 (57)  | 1,722 (37)  | 278 (6) | 24.1 (23.3-25.0) | 1.09 (1.02-1.15) | P = 0.006* |
| ≥ 25 kg/m² | 10,219 (5,887/4,332) | T/C      | 5,651 (56)  | 3,915 (38)  | 653 (6) | 278 (6) | 25.5 (24.9-26.1) | 1.12 (1.04-1.20) | P = 0.003* |
| ≥ 30 kg/m² | 3,913 (1,988/1,925) | C/C      | 2,136 (55)  | 1,507 (38)  | 270 (7) | 27.9 (24.3-31.8) | 1.20 (0.99-1.45) | P = 0.06 |
| ≥ 40 kg/m² | 283 (89/194) |          |              |              |        |        |                   |                   |

| rs17700633 | G/G G/A A/A |
|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
Variants near $MC4R$ associate with obesity

| BMI | G/G | G/A | A/A |  |
|-----|-----|-----|-----|---|
| $< 25 \text{ kg/m}^2$ | 4,694 | 2,511 (54) | 1,848 (39) | 335 (7) | 26.8 (25.9-27.7) |
| $\geq 25 \text{ kg/m}^2$ | 10,174 | 5,073 (50) | 4,276 (42) | 825 (8) | 29.1 (28.5-29.8) |
| $\geq 30 \text{ kg/m}^2$ | 3,886 | 1,922 (49) | 1,631 (42) | 333 (9) | 29.6 (28.5-30.6) |
| $\geq 40 \text{ kg/m}^2$ | 282 (88/194) | 130 (46) | 126 (45) | 26 (9) | 31.6 (27.7-32.5) |

| Waist | G/G | G/A | A/A |  |
|-----|-----|-----|-----|---|
| $< 80 \text{ cm (w)}$ or $< 94 \text{ cm (m)}$ | 5,346 | 2,575 (48) | 2,319 (43) | 452 (9) | 30.1 (29.3-31.0) |
| $\geq 80 \text{ cm (w)}$ or $\geq 94 \text{ cm (m)}$ | 9,467 | 4,394 (47) | 4,104 (43) | 969 (10) | 31.9 (31.2-32.6) |

rs12970134

| BMI | G/G | G/A | A/A |  |
|-----|-----|-----|-----|---|
| $< 25 \text{ kg/m}^2$ | 4,694 | 2,511 (54) | 1,848 (39) | 335 (7) | 26.8 (25.9-27.7) |
| $\geq 25 \text{ kg/m}^2$ | 10,174 | 5,073 (50) | 4,276 (42) | 825 (8) | 29.1 (28.5-29.8) |
| $\geq 30 \text{ kg/m}^2$ | 3,886 | 1,922 (49) | 1,631 (42) | 333 (9) | 29.6 (28.5-30.6) |
| $\geq 40 \text{ kg/m}^2$ | 282 (88/194) | 130 (46) | 126 (45) | 26 (9) | 31.6 (27.7-32.5) |
Variants near *MC4R* associate with obesity

35.6) \( P = 0.02^* \)

| Waist | C/C | C/A | A/A |
|-------|-----|-----|-----|
| < 80 cm (w) or < 94 cm (m) | 5,333 | 2,843 (53) | 2,104 (40) | 386 (7) | 27.0 (26.1-27.8) |
| ≥ 80 cm (w) or ≥ 94 cm (m) | 9,515 | 4,726 (50) | 4,016 (42) | 773 (8) | 29.2 (28.6-29.9) | \( P = 3 \times 10^{-5}^* \) |
| ≥ 88 cm (w) or ≥ 102 cm (m) | 5,710 | 2,831 (50) | 2,412 (42) | 467 (8) | 29.3 (28.5-29.9) | \( P = 4 \times 10^{-4}^* \) |

| rs502933 | C/C | C/A | A/A |
|----------|-----|-----|-----|
| BMI     |     |     |     |
| < 25 kg/m² | 4,637 | 2,002 (43) | 2,067 (45) | 568 (12) | 34.5 (33.6-35.5) |
| ≥ 25 kg/m² | 10,075 | 4,116 (41) | 4,678 (46) | 1,281 (13) | 35.9 (35.3-36.6) | \( P = 0.01 \) |
| ≥ 30 kg/m² | 3,851 | 1,562 (41) | 1,786 (46) | 503 (13) | 36.3 (35.2-37.3) | \( P = 0.01 \) |
| ≥ 40 kg/m² | 281 (91/190) | 104 (37) | 136 (48) | 41 (15) | 38.8 (34.7-43.0) | \( P = 0.04 \) |

| Waist | C/C | C/A | A/A |
|-------|-----|-----|-----|
| < 80 cm (w) or < 94 cm (m) | 5,275 | 2,284 (43) | 2,346 (45) | 645 (12) | 34.5 (33.6-35.4) |
| ≥ 80 cm (w) or ≥ 94 cm (m) | 9,418 | 3,824 (41) | 4,393 (47) | 1,201 (13) | 36.1 (35.4-36.8) | \( P = 0.006^* \) |
| ≥ 88 cm (w) or ≥ 102 cm (m) | 5,662 | 2,281 (40) | 2,666 (47) | 715 (13) | 36.2 (35.3-36.3) | \( P = 0.011^* \) |
Variants near *MC4R* associate with obesity

| rs4450508 | G/G | G/A | A/A |
|-----------|-----|-----|-----|
| **BMI**   |     |     |     |
| < 25 kg/m² | 4,644 | 1,912 (41) | 2,100 (45) | 632 (14) | 36.2 (35.2-37.2) |
| ≥ 25 kg/m² | 10,145 | 3,988 (39) | 4,781 (47) | 1,376 (14) | 37.1 (36.5-37.8) | *P* = 0.08 |
| ≥ 30 kg/m² | 3,873 | 1,510 (39) | 1,820 (47) | 543 (14) | 37.5 (36.4-38.6) | *P* = 0.06 |
| ≥ 40 kg/m² | 282 (86/196) | 100 (36) | 140 (49) | 42 (15) | 39.7 (35.7-43.9) | *P* = 0.09 |

| **Waist** |     |     |     |
| < 80 cm (w) or < 94 cm (m) | 5,293 | 2,192 (41) | 2,391 (45) | 710 (13) | 36.0 (35.1-36.9) |
| ≥ 80 cm (w) or ≥ 94 cm (m) | 9,477 | 3,700 (39) | 4,484 (47) | 1,239 (14) | 37.3 (36.6-38.0) | *P* = 0.03 |
| ≥ 88 cm (w) or ≥ 102 cm (m) | 5,692 | 2,209 (39) | 2,709 (47) | 774 (14) | 37.4 (36.5-38.3) | *P* = 0.05 |

Data are number of individuals with each genotype (% of each group), percentage frequencies of the minor allele (MAF) (95% CI), and odds ratio (OR) (95% CI). Differences in genotype distribution between individuals with a BMI below 25 kg/m², and above 25, 30, or 40 kg/m², respectively; or individuals with a waist circumference below 80 cm (w) or 94 cm (m), and above 80 cm (w) or 94 cm (m), or 88 cm (w) or 102 cm (m), respectively, were calculated applying an additive logistic regression model, while adjusting for sex and age. *P*-values remained significant after Benjamini-Hochberg correction.
Table 3

Genotype distributions, minor allele frequencies and odds ratios for rs17782313, rs17700633, rs12970134, rs502933, and rs4450508 near MC4R among 8,821 individuals with normal glucose tolerance (NGT) and patients with type 2 diabetes.

|                  | n (m/w) | Genotype | MAF (95% CI) | Additive model† OR (95% CI) | Additive model‡ OR (95% CI) |
|------------------|---------|----------|--------------|-----------------------------|-----------------------------|
| rs17782313       |         |          |              |                             |                             |
| NGT              | 4,918   | 2,802 (57) | 1,810 (37) | 306 (6) | 24.6 (23.8-25.5) | 26.1 (25.1-27.1) |
|                  |         | (2,276/2,642) |          |              | 1.08 (0.99-1.18) | 1.00 (0.91-1.09) |
| Type 2 diabetes  | 3,903   | 2,135 (55) | 1,500 (38) | 268 (7) | 26.1 (25.1-27.1) | 1.08 (1.00-1.18) |
|                  |         | (2,317/1,586) |          |              | 1.05 (0.97-1.14) | 1.03 (0.93-1.09) |
|                  |         |           |              |                             |                             |
| rs17700633       |         |          |              |                             |                             |
| NGT              | 4,911   | 2,349 (48) | 2,114 (43) | 448 (9) | 30.6 (29.7-31.6) | 31.2 (30.2-32.2) |
|                  |         | (2,283/2,628) |          |              | 1.05 (0.97-1.14) | 1.03 (0.93-1.09) |
| Type 2 diabetes  | 3,881   | 1,841 (47) | 1,658 (43) | 382 (10) | 31.2 (30.2-32.2) | 1.05 (0.97-1.09) |
|                  |         | (2,305/1,576) |          |              | 1.14 (1.09) | 1.09 |
|                  |         |           |              |                             |                             |
| rs12970134       |         |          |              |                             |                             |
| NGT              | 4,882   | 2,544 (52) | 1,945 (40) | 393 (8) | 28.0 (27.1-28.9) | 29.2 (28.2-30.2) |
|                  |         | (2,261/2,621) |          |              | 1.08 (1.00-1.03) | 1.03 (0.93-1.07) |
| Type 2 diabetes  | 3,839   | 1,942 (50) | 1,631 (42) | 320 (8) | 29.2 (28.2-30.2) | 1.08 (1.00-1.03) |
|                  |         | (2,310/1,580) |          |              | 1.18 | 1.13 |
Variants near \textit{MC4R} associate with obesity

\[ P = 0.06 \quad P = 0.57 \]

| rs502933 | C/C | C/A | A/A |
|----------|-----|-----|-----|
| NGT      | 4,843 | 2,041 (42) | 2,165 (45) | 657 (13) | 35.5 (34.6-36.5) |
|          | (2,244/2,599) | (36.5) |
| Type 2   | 3,834 | 1,518 (40) | 1,822 (47) | 494 (13) | 36.6 (35.6-36.6) |
| diabetes | (2,270/1,561) | (37.7) | 1.16 | 1.14 |

\[ P = 0.11 \quad P = 0.34 \]

| rs4450508 | G/G | G/A | A/A |
|-----------|-----|-----|-----|
| NGT       | 4,845 | 1,975 (41) | 2,183 (45) | 687 (14) | 36.7 (35.7-36.7) |
|           | (2,253/2,592) | (37.7) |
| Type 2    | 3,860 | 1,460 (38) | 1,857 (48) | 543 (14) | 38.1 (37.0-39.2) |
| diabetes  | (2,289/1,568) | (39.2) | 1.16 | 1.16 |

\[ P = 0.08 \quad P = 0.16 \]

Data are number of individuals with each genotype (% of each group), percentage frequencies of the minor allele (MAF) (95% CI), and odds ratio (OR) (95% CI). \( P \)-values compare differences in genotype distribution between normal glucose-tolerant individuals (NGT) and patients with type 2 diabetes applying an additive logistic regression model, while adjusting for sex and age (†) or sex, age and BMI (‡).
Variants near *MC4R* associate with obesity

**Fig. 1 Legend**

Fig. 1. Additive effect of rs12970134 near *MC4R* and *FTO* rs9939609 genotypes on BMI (*n*=5,625). *A*) Values are means; SE’s 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*=1×10$^{-11}$ for additivity).