Association of variants in the PCSK1 gene with obesity in the EPIC-Norfolk study

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Recently, the rs6232 (N221D) and rs6235 (S690T) SNPs in the PCSK1 gene were associated with obesity in a meta-analysis comprising more than 13,000 individuals of European ancestry. Each additional minor allele of rs6232 or rs6235 was associated with a 1.34- or 1.22-fold increase in the risk of obesity, respectively. So far, only one relatively small study has aimed to replicate these findings, but could not confirm the association of the rs6235 SNP and did not study the rs6232 variant. In the present study, we examined the associations of the rs6232 and rs6235 SNPs with obesity in a population-based cohort consisting of 20,249 individuals of European descent from Norfolk, UK. Logistic regression and generalized linear models were used to test the associations of the risk alleles with obesity and related quantitative traits, respectively. Neither of the SNPs was significantly associated with obesity, BMI or waist circumference under the additive genetic model \( P > 0.05 \). However, we observed an interaction between rs6232 and age on the level of BMI \( P = 0.010 \) and risk of obesity \( P = 0.020 \). The rs6232 SNP was associated with BMI \( P = 0.021 \) and obesity \( P = 0.022 \) in the younger individuals [less than median age (59 years)], but not among the older age group \( P = 0.81 \) and \( P = 0.68 \) for BMI and obesity, respectively. In conclusion, our data suggest that the PCSK1 rs6232 and rs6235 SNPs are not major contributors to common obesity in the general population. However, the effect of rs6232 may be age-dependent.

INTRODUCTION

Obesity is a leading risk factor for several common diseases such as type 2 diabetes, cardiovascular disease and cancer (1). As its prevalence keeps growing rapidly in both developed and developing countries, the prevention of obesity is now a major challenge for clinicians and public health policy makers worldwide (2–4). Obesity has a strong genetic basis (5,6), but the detection of specific genetic risk variants has proven difficult. Recently, genome-wide association studies have been successful in identifying at least 16 obesity susceptibility loci (7–12). Despite robust associations, these loci explain less than 2% of the individual variation in the risk of obesity (11). Furthermore, for most of these loci, the functional role remains to be elucidated.

The candidate gene approach, where promising genes are investigated on the basis of etiological understanding of disease, has not been very successful in identifying obesity susceptibility genes. Recently, however, Benzinou et al. (13) was able to detect highly significant associations between the non-synonymous rs6232 (N221D) and rs6235 (S690T) SNPs in the prohormone convertase 1/3 (PCSK1) gene and the risk of obesity by using a staged, comprehensive candidate gene approach. After a systematical screening of the PCSK1 gene for common variants, Benzinou et al. followed up the obesity-associated rs6232 and rs6235 SNPs in altogether seven case control studies and one family study, comprising a total of 13,659 individuals of European ancestry. Finally, a meta-analysis of seven of the included cohorts showed that each additional minor allele of rs6232 or rs6235 was associated with

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a 1.34-fold \( (P = 7.3 \times 10^{-8}) \) or 1.22-fold \( (P = 2.3 \times 10^{-12}) \)
increase in the risk of obesity, respectively (13).

The \( PCSK1 \) gene encodes the prohormone convertase 1/3 enzyme, expressed in neuroendocrine cells, that converts pro-
hormones into functional hormones which regulate energy
metabolism. Mutations in the \( PCSK1 \) gene have been found to
cause monogenic obesity (14,15,16). Furthermore, four
genome-wide linkage studies have suggested an association
with the common 5.6 Mb interval on chromosome 5q contain-
ing the \( PCSK1 \) gene and obesity-associated traits (17–20).

The N221D substitution, encoded by rs6232, is located in
the catalytic domain of prohormone convertase 1/3, immedi-
ately adjacent to the N222D substitution, which leads to
maturity-onset obesity and increased body fat content in
homozygous mutant mice (21). The N221D substitution is
also located near to two other polymorphic sites (Q250stop
and A213del) which, respectively, truncate the prohormone
convertase and delete a highly conserved alanine residue
near the catalytically critical His208 residue (15). In vitro,
N221D substitution moderately reduces the activity of the pro-
hormone convertase (13).

The rs6235 SNP, encoding S690T, is highly correlated with
another non-synonymous SNP rs6234 that encodes Q665E. Both
substitutions are located in the C-terminal region of the
protein, which has been shown to be important for correct
targeting and specificity of the prohormone convertase 1/3 and its
sorting in secretory granules (22). The Q665E–S690T cluster
has not, however, been found to alter the activity of the con-
vertase (13,16) or its maturation and secretion (13).

A recent meta-analysis of genome-wide association studies
in a total of 32 387 individuals found a weak association of
rs6232 with BMI \( (P = 0.03 \) in the appropriate direction),
whereas no data was available for rs6234/rs6235 or for
obesity risk as an outcome (11). A replication in the
population-based Northern Swedish Health and Disease
(NSHED) study could not confirm association between
rs6235 and obesity [odds ratio (OR) 1.05, \( P = 0.30 \)] among
1723 non-diabetic Swedes (23), and no data for the rs6232
SNP was reported. The findings thus require further replication
in large-scale populations sufficiently powered to identify the
initially observed effect sizes.

The aim of this study was to investigate whether the rs6232
and rs6235 polymorphisms in the \( PCSK1 \) gene are associated
with obesity and obesity-related phenotypes in a population-
based cohort comprising 20 249 individuals of European
descent from Norfolk, UK.

RESULTS

The minor alleles of rs6232 and rs6235 SNPs in the \( PCSK1 \)
gene were not associated with an increased risk of obesity in
comparisons between obese versus non-obese individuals or
between obese versus normal-weight individuals in any of
the tested obesity categories under the additive genetic
model (Tables 1 and 2). Consistently, no associations were
observed between the rs6232 and rs6235 SNPs and the
obesity-related quantitative traits (BMI, waist circumference)
(Table 3). None of the associations were attenuated by the
individuals’ physical activity levels \( (P_{\text{interaction}} > 0.10) \).

We found a significant interaction between the rs6232 SNP
and age on the risk of obesity in comparisons between obese
versus normal weight individuals \( (P = 0.020) \). Therefore, we
additionally analysed the association of the rs6232 SNP with
obesity in the younger and older individuals separately, strati-
fied by the median age (59 years) of the cohort. The rs6232
SNP was associated with obesity in the younger age group
\( (OR = 1.24, 95\% \ CI 1.03–1.50; \ P = 0.022) \), but not among
the older individuals \( (OR = 0.96, 95\% \ CI 0.80–1.16; \ P =
0.68) \) (Table 2). Consistently, we found a significant
interaction between age and the rs6235 SNP on the level of BMI
\( (P = 0.010) \). The rs6232 SNP was associated with BMI
among the younger individuals \( (\beta = 0.28 \pm 0.12, \ P =
0.021) \), but not in the older age group \( (\beta = -0.03 \pm 0.11;
\ P = 0.81) \) (Table 3). No significant interaction between age
and the rs6235 SNP on the level of obesity as a binary trait
was found in comparisons between obese versus non-obese
\( (P = 0.43) \) or obese versus normal weight \( (P = 0.58) \)
individuals. The rs6235 SNP did neither interact with age on BMI
\( (P = 0.75) \) or waist circumference \( (P = 0.65) \).

No significant interactions between the rs6232 or rs6235
SNPs and sex were found \( (P_{\text{interaction}} > 0.05 \) in all tested
models). However, when comparing obese versus normal
weight individuals, the rs6235 minor allele was associated
with a higher risk of obesity in women \( (OR = 1.10, 95\% \ CI
1.01–1.03; \ P = 0.046) \), but not in men \( (OR = 1.02, 95\% \ CI
1.01–1.03; \ P = 0.76) \). We also found a significant association
of the rs6232 SNP with waist circumference in women \( (\beta =
0.74 \pm 0.33; \ P = 0.023) \), but not in men \( (\beta = 0.053 \pm 0.29;
\ P = 0.86) \). No other significant associations of the rs6232 or
rs6235 SNPs with obesity or associated traits were found in
men or women.

Our study had a 99.9% power to detect OR 1.34 with rs6232
and OR 1.22 with rs6235 when comparing class I obese
\( (BMI \geq 30 \text{ kg/m}^2) \) individuals to non-obese \( (BMI < 30 \text{ kg/}
\text{m}^2) \) individuals under an additive model and significance
level of 0.05. Thus, our analyses had sufficient power to
detect similar effect sizes as previously reported using the
class I obesity threshold. (13). The power to detect the ORs
1.34 and 1.22 with rs6232 and rs6235 in the comparisons
between class II obese \( (BMI \geq 35 \text{ kg/m}^2) \) and non-obese
individuals were 63.2% for rs6232 and 33.0% for rs6235. The
power to detect the same ORs when comparing class III
obese \( (BMI \geq 40 \text{ kg/m}^2) \) individuals to non-obese individuals
was 20.0 and 11.4% for rs6232 and rs6235, respectively.

To provide a pooled OR for the association of the rs6235
polymorphism with obesity in a total of 12 439 individuals
included in the present study and the NSHED study (23), we
performed a meta-analysis by the inverse variance method.
We used ORs derived from comparisons between obese and
normal weight individuals because they were available from
both studies. We observed a non-significant OR of 1.05
\( (95\% \ CI 0.99–1.11; \ P = 0.11) \). Additionally, we performed
a meta-analysis to provide a pooled effect size for the associ-
ation of the rs6232 SNP with BMI in the present study
and in the 32 387 individuals of the recent genome-wide
meta-analysis on BMI (11). As there was an overlap of 2168
individuals between the present study and the genome-wide
meta-analysis, these individuals were removed from the
present data while calculating effect size for the meta-analysis.
Furthermore, the \( P \)-value and its standard error for the association of the rs6232 SNP with BMI were not available in the publication by Willer et al. (11), and they were thus estimated on the basis of the association \( P \)-value and number of individuals. The pooled effect size we calculated for the rs6232 SNP among the total of 50,468 individuals was 0.17 kg/m\(^2\) per allele (\( P = 0.004 \)).

### DISCUSSION

Recently, a meta-analysis comprising more than 13,000 individuals from one family-based and seven case–control cohorts found significant association of the rs6232 (N221D) and rs6235 (S690T) SNPs in the \( PCSK1 \) gene with the risk of obesity. The present study aimed to replicate these findings in a large population-based cohort of European ancestry from Norfolk, UK. Despite the fact that we had sufficient power to replicate the previously reported results, we did not detect significant associations between the rs6232 and rs6235 polymorphisms and obesity or obesity-related phenotypes. However, we observed interactions between the rs6232 SNP and age on the level of obesity and BMI. The rs6232 SNP was associated with obesity only among the younger age group (less than 59 years), but not in the older individuals.

The discrepant findings between the present study and Benzinou et al. (13) may in part be explained by differences in the age of the studied individuals. The study by Benzinou et al. included three case–control cohorts of children, in whom genetic influences on BMI may be stronger than in

### Table 1. Association of the rs6232 and rs6235 SNPs with obesity

| rs6232                  |     |     |     | Odds ratio (95% CI) | \( P \)-value |
|-------------------------|-----|-----|-----|--------------------|--------------|
|                         | AA  | AG  | GG  |                    |              |
| Non-obese (BMI < 30 kg/m\(^2\)) | 15   | 461 |     | 1.05 (0.93–1.19)   | 0.41         |
| Obese (BMI \( \geq 30 \text{kg/m}^2 \)) | 26   | 62 |     | 0.97 (0.73–1.28)   | 0.81         |
| Class II obese (BMI \( \geq 35 \text{kg/m}^2 \)) | 465  | 48 | 3   | 1.11 (0.65–1.91)   | 0.70         |
| Class III obese (BMI \( \geq 40 \text{kg/m}^2 \)) | 104  | 14 | 0   |                    |              |
| rs6235                  |     |     |     |                    |              |
|                         | GG  | GC  | CC  |                    |              |
| Non-obese (BMI < 30 kg/m\(^2\)) | 9074 | 6764 |   | 1.03 (0.96–1.09)   | 0.42         |
| Obese (BMI \( \geq 30 \text{kg/m}^2 \)) | 1521 | 1202 | 203 |                    |              |
| Class II obese (BMI \( \geq 35 \text{kg/m}^2 \)) | 278  | 198 | 39  | 1.00 (0.87–1.15)   | 0.98         |
| Class III obese (BMI \( \geq 40 \text{kg/m}^2 \)) | 59   | 48 | 11  | 1.16 (0.87–1.53)   | 0.31         |

Obese versus non-obese individuals. All odds ratios and \( P \)-values are calculated with non-obese individuals as the reference group and are adjusted for age and sex. \( P \)-values are for the additive genetic model.

### Table 2. Association of the rs6232 and rs6235 SNPs with obesity

| rs6232                  |     |     |     | Odds ratio (95% CI) | \( P \)-value |
|-------------------------|-----|-----|-----|--------------------|--------------|
|                         | AA  | AG  | GG  |                    |              |
| All subjects            |     |     |     |                    |              |
| Normal weight (BMI < 25 kg/m\(^2\)) | 7113 | 802 | 18  |                    |              |
| Obese (BMI \( \geq 30 \text{kg/m}^2 \)) | 2612 | 311 | 13  | 1.09 (0.95–1.24)   | 0.22         |
| Class II obese (BMI \( \geq 35 \text{kg/m}^2 \)) | 465  | 48  | 3   | 1.00 (0.75–1.33)   | 1.00         |
| Class III obese (BMI \( \geq 40 \text{kg/m}^2 \)) | 104  | 14 | 0   | 1.16 (0.67–2.01)   | 0.60         |
| Age \( < 59 \) years    |     |     |     |                    |              |
| Normal weight (BMI < 25 kg/m\(^2\)) | 3946 | 424 | 9   |                    |              |
| Obese (BMI \( \geq 30 \text{kg/m}^2 \)) | 1172 | 157 | 4   | 1.24 (1.03–1.50)   | 0.022        |
| Class II obese (BMI \( \geq 35 \text{kg/m}^2 \)) | 220  | 29  | 1   | 1.25 (0.85–1.82)   | 0.26         |
| Class III obese (BMI \( \geq 40 \text{kg/m}^2 \)) | 54   | 9 | 0   | 1.45 (0.72–2.90)   | 0.29         |
| Age \( \geq 59 \) years |     |     |     |                    |              |
| Normal weight (BMI < 25 kg/m\(^2\)) | 3167 | 378 | 9   |                    |              |
| Obese (BMI \( \geq 30 \text{kg/m}^2 \)) | 1440 | 154 | 9   | 0.96 (0.80–1.16)   | 0.68         |
| Class II obese (BMI \( \geq 35 \text{kg/m}^2 \)) | 245  | 19  | 2   | 0.79 (0.51–1.22)   | 0.29         |
| Class III obese (BMI \( \geq 40 \text{kg/m}^2 \)) | 50   | 5 | 0   | 0.86 (0.35–2.12)   | 0.74         |
| rs6235                  |     |     |     |                    |              |
|                         | GG  | GC  | CC  |                    |              |
| All subjects            |     |     |     |                    |              |
| Normal weight (BMI < 25 kg/m\(^2\)) | 4197 | 3038 | 558 |                    |              |
| Obese (BMI \( \geq 30 \text{kg/m}^2 \)) | 1521 | 1202 | 203 |                    |              |
| Class II obese (BMI \( \geq 35 \text{kg/m}^2 \)) | 278  | 196 | 39  | 1.01 (0.88–1.17)   | 0.86         |
| Class III obese (BMI \( \geq 40 \text{kg/m}^2 \)) | 59   | 48 | 11  | 1.17 (0.88–1.55)   | 0.27         |

Obese versus normal weight individuals. All odds ratios and \( P \)-values are calculated with normal weight individuals as the reference group and assuming additive genetic model. The \( P \)-values for the analyses in all subjects are adjusted for age and sex. The \( P \)-values for the analyses in age subgroups are adjusted for sex only. \( P = 0.020 \), \( P = 0.065 \) and \( P = 0.32 \) for the interaction between rs6232 and age (modelled as a continuous variable) on obesity, class II obesity and class III obesity, respectively.
adults (24). The present study only included individuals of 40–79 years of age, but the interaction of the rs6232 SNP with age suggests that rs6232 SNP may be associated with obesity more strongly or exclusively among younger age groups. Furthermore, a high number of the obese cases studied by Benzinou et al. (13) were class III (BMI ≥ 40 kg/m²) obese, whereas only 4% of the obese participants of the present study had such a high BMI. Severely and morbidly obese individuals are likely to carry more genetic risk variants for obesity than other individuals, and thus a high number of such individuals may lead to inflated ORs in genetic association studies (13). It is also possible that the associations of the rs6232 and rs6235 SNPs become apparent only among individuals with severe obesity, and our study was underpowered to detect such effects. Indeed, the ORs were strongest when class III obese individuals were compared with either non-obese or normal weight individuals (Tables 1 and 2).

Consistently with the present study, a replication in the population-based NSHED study did not find a significant association for the rs6235 polymorphism with obesity among 1723 individuals (OR 1.05; P = 0.30) or BMI among 3885 individuals (P = 0.59) (23). The pooled OR that we calculated on the basis of the available data did not suggest a significant association between the rs6235 variant and obesity. Our meta-analysis on the rs6232 SNP among more than 50 000 individuals, showed a modest association with BMI. The effect size we meta-analysed for the rs6232 SNP was 0.17 kg/m² per allele, which is approximately half of the effect size of the rs9939609 SNP in the FTO gene, the strongest common obesity risk variant known so far (11). The minor allele frequency (MAF) of the rs6232 SNP is, however, low (5%), and rs6232 is thus likely to provide a rather weak population-attributable risk on common obesity. Thus, the rs6232 SNP seems not to be a major contributor to obesity in the general population.

In summary, although we found an effect in the appropriate direction, our findings from the population-based EPIC-Norfolk study do not support that the rs6232 and rs6235 SNPs of the PCSK1 gene are major contributors to common obesity in the general population. Further studies are warranted to examine whether common variants in the PCSK1 gene contribute to increased risk of obesity in other populations, especially among younger age groups.

### MATERIALS AND METHODS

The study population included 20 249 individuals (9998 men, 10 251 women) from the EPIC-Norfolk cohort, a population-based study of men and women aged 40–79 years and recruited in Norfolk, UK. The study design, methods and measurements have been described in detail previously (25). In brief, all participants attended a clinical examination that included standard anthropometric measurements. Height and weight were measured with participants dressed in lightweight clothing without shoes, and BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured midway between the lowest rib and the iliac crest to the nearest millimeter at the end of expiration. Habitual physical activity was assessed using two questions referring to activity during the past year, described in detail elsewhere (26). On the basis of these questions, the individuals were allocated to four categories: inactive, moderately inactive, moderately active and active. The method has been validated.

### Table 3. Association of the rs6232 and rs6235 SNPs with obesity-related quantitative traits

| rs6232 | All subjects | AA | AG | GG | β ± SE | P-value |
|--------|--------------|----|----|----|--------|---------|
| BMI (kg/m²) | 26.31 ± 0.03 | 26.38 ± 0.08 | 27.46 ± 0.47 | 0.12 ± 0.08 | 0.13 |
| Waist (cm) | 88.89 ± 0.08 | 89.12 ± 0.22 | 91.83 ± 1.25 | 0.38 ± 0.22 | 0.082 |
| Hip (cm) | 103.04 ± 0.06 | 103.09 ± 0.17 | 105.36 ± 0.97 | 0.18 ± 0.17 | 0.28 |
| Waist-to-hip | 0.862 ± 0.000 | 0.864 ± 0.001 | 0.871 ± 0.007 | 0.002 ± 0.001 | 0.092 |

### rs6235

| All subjects | GG | GC | CC | β ± SE | P-value |
|--------------|----|----|----|--------|---------|
| BMI (kg/m²) | 26.29 ± 0.04 | 26.41 ± 0.04 | 26.32 ± 0.10 | 0.07 ± 0.04 | 0.12 |
| Waist (cm) | 88.83 ± 0.10 | 89.07 ± 0.11 | 89.20 ± 0.27 | 0.21 ± 0.11 | 0.064 |
| Hip (cm) | 102.99 ± 0.08 | 103.18 ± 0.09 | 103.13 ± 0.21 | 0.13 ± 0.09 | 0.14 |
| Waist-to-hip | 0.862 ± 0.001 | 0.862 ± 0.001 | 0.864 ± 0.002 | 0.001 ± 0.001 | 0.16 |

Data are mean ± SE. β indicates the difference in trait per copy of the risk allele. P-values are for the additive genetic model. Analyses in all subjects are adjusted for age and sex. Analyses in age subgroups are adjusted for sex only. P-values for the interactions between rs6232 and age (modelled as a continuous variable) on BMI, waist circumference, hip circumference and waist-to-hip ratio were 0.010, 0.055, 0.13 and 0.10, respectively.
and class III obese (BMI ≥ 40 kg/m²) categories separately. Physical activity and age were entered into the models as continuous variables. All analyses were adjusted for age and sex, and the reported P-values are nominal and two sided. Power calculations were performed using the Quanto software (http://hydra.usc.edu/gxe). A likelihood ratio test was performed to assess Hardy–Weinberg equilibrium. Statistical analyses were performed with SAS 9.1 (SAS Institute, Cary, NC, USA) except for the meta-analysis, which was carried out with Stata 10.1 (Stata Corporation LP, College Station, TX, USA). The inverse variance method was used to pool ORs or betas from individual studies.

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Table 4. Participant characteristics

|                  | Men     | Women  |
|------------------|---------|--------|
| n                | 9998    | 10 251 |
| Age (years)      | 59.1 ± 9.3 | 58.6 ± 9.3 |
| BMI (kg/m²)      | 26.5 ± 3.3 | 26.1 ± 4.2 |
| Waist circumference (cm) | 95.7 ± 9.7 | 82.1 ± 10.8 |
| Hip circumference (cm) | 102.7 ± 6.3 | 103.4 ± 9.1 |
| Waist-to-hip ratio | 0.93 ± 0.06 | 0.79 ± 0.06 |
| Normal weight (BMI < 25 kg/m²) | 3330 | 4603 |
| Overweight (BMI 25–30 kg/m²) | 5363 | 4017 |
| Class I obese (BMI 30–35 kg/m²) | 1160 | 1260 |
| Class II obese (BMI 35–40 kg/m²) | 122 | 276 |
| Class III obese (BMI ≥ 40 kg/m²) | 23 | 95 |

Data are mean ± SD.
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