Human Molecular Genetics, 2009, Vol. 18, No. 18 **3496–3501**
doi:10.1093/hmg/ddp280
Advance Access published on June 15, 2009

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

Tuomas O. Kilpeläinen[1,*], Sheila A. Bingham[2], Kay-Tee Khaw[3], Nicholas J. Wareham[1] and Ruth J.F. Loos[1]

[1]MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge CB2 0QQ, UK, [2]CNC, Department of Public Health and Primary Care, University of Cambridge, Cambridge CB1 8RN, UK and [3]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge CB2 0SR, UK

Received April 1, 2009; Revised May 19, 2009; Accepted June 11, 2009

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

*To whom correspondence should be addressed at: MRC Epidemiology Unit, Institute of Metabolic Science, Box 285, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, UK. Tel: +44 1223769164; Fax: +44 1223330316; Email: tuomas.kilpelainen@mrc-epid.cam.ac.uk

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 prohormones 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 containing 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, immediately 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 prohormone 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 convertase (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, stratified 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 rs6232 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 1.00–1.20; $P = 0.046$), 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 ≥ 30 kg/m^2) individuals to non-obese (BMI < 30 kg/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 ≥ 35 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 ≥ 40 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 association 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.

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

				Odds ratio (95% CI)	*P*-value
rs6232	AA	AG	GG		
Non-obese (BMI < 30 kg/m^2)	15 461	1800	52		
Obese (BMI ≥ 30 kg/m^2)	2612	311	13	1.05 (0.93–1.19)	0.41
Class II obese (BMI ≥ 35 kg/m^2)	465	48	3	0.97 (0.73–1.28)	0.81
Class III obese (BMI ≥ 40 kg/m^2)	104	14	0	1.11 (0.65–1.91)	0.70
rs6235	GG	GC	CC		
Non-obese (BMI < 30 kg/m^2)	9074	6764	1228		
Obese (BMI ≥ 30 kg/m^2)	1521	1202	203	1.03 (0.96–1.09)	0.42
Class II obese (BMI ≥ 35 kg/m^2)	278	198	39	1.00 (0.87–1.15)	0.98
Class III obese (BMI ≥ 40 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

				Odds ratio (95% CI)	*P*-value
rs6232	AA	AG	GG		
All subjects					
Normal weight (BMI < 25 kg/m^2)	7113	802	18		
Obese (BMI ≥ 30 kg/m^2)	2612	311	13	1.09 (0.95–1.24)	0.22
Class II obese (BMI ≥ 35 kg/m^2)	465	48	3	1.00 (0.75–1.33)	1.00
Class III obese (BMI ≥ 40 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 ≥ 30 kg/m^2)	1172	157	4	1.24 (1.03–1.50)	0.022
Class II obese (BMI ≥ 35 kg/m^2)	220	29	1	1.25 (0.85–1.82)	0.26
Class III obese (BMI ≥ 40 kg/m^2)	54	9	0	1.45 (0.72–2.90)	0.29
Age ≥ 59 years					
Normal weight (BMI < 25 kg/m^2)	3167	378	9		
Obese (BMI ≥ 30 kg/m^2)	1440	154	9	0.96 (0.80–1.16)	0.68
Class II obese (BMI ≥ 35 kg/m^2)	245	19	2	0.79 (0.51–1.22)	0.29
Class III obese (BMI ≥ 40 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 ≥ 30 kg/m^2)	1521	1202	203	1.05 (0.98–1.12)	0.20
Class II obese (BMI ≥ 35 kg/m^2)	278	198	39	1.01 (0.88–1.17)	0.86
Class III obese (BMI ≥ 40 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.

Furthermore, the β-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 3. Association of the rs6232 and rs6235 SNPs with obesity-related quantitative traits

				$\beta \pm$ SE	*P*-value
rs6232	AA	AG	GG		
All subjects					
BMI (kg/m^2)	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
Age <59 years					
BMI (kg/m^2)	26.00 ± 0.04	26.25 ± 0.12	26.95 ± 0.73	0.28 ± 0.12	0.021
Waist (cm)	87.01 ± 0.11	87.52 ± 0.31	89.49 ± 1.91	0.58 ± 0.32	0.067
Hip (cm)	102.62 ± 0.08	102.88 ± 0.24	104.17 ± 1.48	0.31 ± 0.25	0.21
Waist-to-hip	0.847 ± 0.001	0.850 ± 0.002	0.859 ± 0.011	0.003 ± 0.002	0.076
Age ≥59 years					
BMI (kg/m^2)	26.63 ± 0.04	26.50 ± 0.11	27.87 ± 0.61	−0.03 ± 0.11	0.81
Waist (cm)	90.73 ± 0.11	90.70 ± 0.31	93.78 ± 1.66	0.15 ± 0.30	0.61
Hip (cm)	103.44 ± 0.08	103.30 ± 0.24	106.31 ± 1.29	0.06 ± 0.24	0.80
Waist-to-hip	0.877 ± 0.001	0.877 ± 0.002	0.883 ± 0.010	0.001 ± 0.002	0.64
rs6235	GG	GC	CC		
All subjects					
BMI (kg/m^2)	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.

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 $\geq$ 40 kg/m^2) 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/m^2 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 4. Participant characteristics

	Men	Women
n	9998	10 251
Age (years)	59.1 ± 9.3	58.6 ± 9.3
BMI (kg/m^2)	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/m^2)	3330	4603
Overweight (BMI 25–30 kg/m^2)	5363	4017
Class I obese (BMI 30–35 kg/m^2)	1160	1260
Class II obese (BMI 35–40 kg/m^2)	122	276
Class III obese (BMI ≥ 40 kg/m^2)	23	95

Data are mean ± SD.

against heart rate monitoring with individual calibration in independent studies (27,28). The study protocol was approved by The Norfolk and Norwich Hospital Ethics Committee, and informed consent was obtained from all participants. Descriptive characteristics of the population are given in Table 4.

Genotyping

Genotyping was performed with Custom TaqMan SNP Genotyping Assays (Applied Biosystems, Warrington, UK). The Assays were carried out on 10 ng of genomic DNA in a 2.5 μl standard 384-well TaqMan assay using a G-Storm GS4 Thermal Cycler (GRI Ltd, Essex, UK), cycling 95°C for 10 min, and then 40 cycles of 15 s at 92°C and 1 min at 54°C. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK) was used for endpoint detection and allele calling. The call rates were 97.7% for rs6232 and 96.4% for rs6235. The 864 duplicate controls used for rs6232 were 98.6% concordant and the 817 duplicates used for rs6235 were 98.8% concordant. The genotypic distributions of the SNPs did not deviate from the Hardy–Weinberg equilibrium ($P = 0.87$ for rs6232; $P = 0.24$ for rs6235). The MAFs observed in this study (5.5% for rs6232 G-allele and 27.1% for rs6235 C-allele) were similar to those reported by Benzinou *et al*. (13).

Statistical analysis

Logistic regression was used to test for the association between the *PCSK1* SNPs and obesity. Comparisons were carried out between obese (BMI ≥ 30 kg/m^2) versus non-obese (BMI < 30 kg/m^2) and between obese versus normal weight (BMI < 25 kg/m^2) individuals separately. In addition, to see whether the association of the *PCSK1* SNPs with obesity depends on the level of BMI, we compared the normal weight and non-obese individuals to each class I obese (BMI ≥ 30 kg/m^2), class II obese (BMI ≥ 35 kg/m^2) and class III obese (BMI ≥ 40 kg/m^2) categories separately. Quantitative traits (BMI and waist circumference) were analysed using generalized linear models among all 20 249 participants. Interactions between the SNPs and physical activity (four categories), sex or age were tested by including a two-way interaction term (SNP*physical activity, SNP*sex or SNP*age) into the main effects model. 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.

ACKNOWLEDGEMENTS

This work was supported by program grants from the Medical Research Council UK and Cancer Research UK; and by additional support from the European Union; Stroke Association; British Heart Foundation; Department of Health; Food Standards Agency; and the Wellcome Trust.

Conflict of Interest statement. None declared.

FUNDING

Funding to pay the Open Access publication charges for this article was provided by MRC Epidemiology Unit.

REFERENCES

1. Must, A., Spadano, J., Coakley, E.H., Field, A.E., Colditz, G. and Dietz, W.H. (1999) The disease burden associated with overweight and obesity. *J. Am. Med. Assoc.*, **282**, 1523–1529.
2. Ford, E.S. and Mokdad, A.H. (2008) Epidemiology of obesity in the Western hemisphere. *J. Clin. Endocrinol. Metab.*, **93**, S1–S8.
3. Misra, A. and Khurana, L. (2008) Obesity and the metabolic syndrome in developing countries. *J. Clin. Endocrinol. Metab.*, **93**, S9–S30.
4. Berghofer, A., Pischon, T., Reinhold, T., Apovian, C.M., Sharma, A.M. and Willich, S.N. (2008) Obesity prevalence from a European perspective: a systematic review. *BMC Public Health*, **8**, 200.
5. Bell, C.G., Walley, A.J. and Froguel, P. (2005) The genetics of human obesity. *Nat. Rev. Genet.*, **6**, 221–234.
6. Lyon, H.N. and Hirschhorn, J.N. (2005) Genetics of common forms of obesity: a brief overview. *Am. J. Clin. Nutr.*, **82**, 215S–217S.
7. Frayling, T.M., Timpson, N.J., Weedon, M.N., Zeggini, E., Freathy, R.M., Lindgren, C.M., Perry, J.R., Elliott, K.S., Lango, H., Rayner, H. *et al.* (2007) A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*, **316**, 889–894.
8. Scuteri, A., Sanna, S., Chen, W.M., Uda, M., Albai, G., Strait, J., Najjar, S., Nagaraja, R., Orru, M., Usala, M. *et al.* (2007) Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet.*, **3**, e115.
9. Loos, R.J., Lindgren, C.M., Li, S., Wheeler, E., Zhao, J.H., Prokopenko, I., Inouye, M., Freathy, R.M., Attwood, A.P., Beckmann, J.S. *et al.* (2008) Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat. Genet.*, **40**, 768–775.
10. Thorleifsson, G., Walters, G.B., Gudbjartsson, D.F., Steinthorsdottir, V., Sulem, P., Helgadottir, A., Styrkarsdottir, U., Gretarsdottir, S., Thorlacius, S., Jonsdottir, I. *et al.* (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat. Genet.*, **41**, 18–24.
11. Willer, C.J., Speliotes, E.K., Loos, R.J., Li, S., Lindgren, C.M., Heid, I.M., Berndt, S.I., Elliott, A.L., Jackson, A.U., Lamina, C. *et al.* (2009) Six new loci associated with body mass index highlight a

neuronal influence on body weight regulation. *Nat. Genet.*, **41**, 25–34.
12. Meyre, D., Delplangue, J., Chevre, J.C., Lecoeur, C., Lobbens, S., Gallina, S., Durand, E., Vatin, V., Degraeve, F., Proenca, C. *et al.* (2009) Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat. Genet.*, **41**, 157–159.
13. Benzinou, M., Creemers, J.W., Choquet, H., Lobbens, S., Dina, C., Durand, E., Guerardel, A., Boutin, P., Jouret, B., Heude, B. *et al.* (2008) Common nonsynonymous variants in PCSK1 confer risk of obesity. *Nat. Genet.*, **40**, 943–945.
14. Jackson, R.S., Creemers, J.W., Ohaqi, S., Raffin-Sanson, M.L., Sanders, L., Montaque, C.T., Hutton, C.T. and O'Rahilly, S. (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat. Genet.*, **16**, 303–306.
15. Jackson, R.S., Creemers, J.W., Farooqi, I.S., Raffin-Sanson, M.L., Varro, A., Dockray, G.J., Holst, J.J., Brubaker, P.L., Corvol, P., Polonsky, K.S. *et al.* (2003) Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J. Clin. Invest.*, **112**, 1550–1560.
16. Farooqi, I.S., Volders, K., Stanhope, R., Heuschkel, R., White, A., Lank, E., Koegh, J., O'Rahilly, S. and Creemers, J.W. (2007) Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *J. Clin. Endocrinol. Metab.*, **92**, 3369–3373.
17. Hager, J., Dina, C., Francke, S., Dubois, S., Houari, M., Vatin, V., Vaillant, E., Lorentz, N., Basdevant, A., Clement, K. *et al.* (1998) A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nat. Genet.*, **20**, 304–308.
18. Chagnon, Y.C., Rice, T., Perusse, L., Borecki, I.B., Ho-Kim, M.A., Lacaille, M., Pare, C., Bouchard, L., Gagnon, J., Leon, A.S. *et al.* (2001) Genomic scan for genes affecting body composition before and after training in Caucasians from HERITAGE. *J. Appl. Physiol.*, **90**, 1777–1787.
19. Bell, C.G., Benzinou, M., Siddiq, A., Lecoeur, C., Dina, C., Lemainque, A., Clement, K., Basdevant, A., Guy-Grand, B., Mein, C.A. *et al.* (2004) Genome-wide linkage analysis for severe obesity in French Caucasians finds significant susceptibility locus on chromosome 19q. *Diabetes*, **53**, 1857–1865.
20. Chen, G., Adeyemo, A.A., Johnson, T., Zhou, J., Amoah, A., Owusu, S., Acheampong, J., Agyenim-Boateng, K., Eghan, B.A., Oli, J. *et al.* (2005) A genome-wide scan for quantitative trait loci linked to obesity phenotypes among West Africans. *Int. J. Obes.*, **29**, 255–259.
21. Lloyd, D.J., Bohan, S. and Gekakis, N. (2006) Obesity, hyperphagia and increased metabolic efficiency in Pc1 mutant mice. *Hum. Mol. Genet.*, **15**, 1884–1893.
22. Zhou, Y. and Lindberg, I. (1994) Enzymatic properties of carboxyl-terminally truncated prohormone convertase 1 (PC1/SPC3) and evidence for autocatalytic conversion. *J. Biol. Chem.*, **269**, 18408–18413.
23. Renström, F., Payne, F., Nordström, A., Brito, E.C., Rolandsson, O., Hallmans, G., Barroso, I., Nordström, P. and Franks, P.W. GIANT Consortium (2009) Replication and extension of genome-wide association study results for obesity in 4,923 adults from Northern Sweden. *Hum. Mol. Genet.*, January 22 [Epub ahead of print].
24. Wardle, J., Carnell, S., Haworth, C.M.A. and Plomin, R. (2008) Evidence for a strong genetic influence on childhood despite the force of the obesogenic environment. *Am. J. Clin. Nutr.*, **87**, 398–404.
25. Day, N., Oakes, S., Luben, R., Khaw, K.T., Bingham, S., Welch, A. and Wareham, N. (1999) EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. *Br. J. Cancer.*, **80** (Suppl. 1), 95–103.
26. McFadden, E., Luben, R., Wareham, N., Bingham, S. and Khaw, K.T. (2008) Occupational social class, risk factors and cardiovascular disease incidence in men and women: a prospective study in the European prospective investigation of cancer and nutrition in Norfolk (EPIC-Norfolk) cohort. *Eur. J. Epidemiol.*, **23**, 449–458.
27. Wareham, N.J., Jakes, R.W., Rennie, K.L., Mitchell, J., Hennings, S. and Day, N.E. (2002) Validity and repeatability of the EPIC-Norfolk Physical Activity Questionnaire. *Int. J. Epidemiol.*, **31**, 168–174. 2002.
28. Wareham, N.J., Jakes, R.W., Rennie, K.L., Schuit, J., Mitchell, J., Hennings, S. and Day, N.E. (2003) Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr.*, **6**, 407–413.