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Original Article

ORIGINAL ARTICLE

Contribution of common non-synonymous variants in *PCSK1* to body mass index variation and risk of obesity: a systematic review and meta-analysis with evidence from up to 331 175 individuals

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Abstract

Polymorphisms rs6232 and rs6234/rs6235 in *PCSK1* have been associated with extreme obesity [e.g. body mass index (BMI) ≥ 40 kg/m^2], but their contribution to common obesity (BMI ≥ 30 kg/m^2) and BMI variation in a multi-ethnic context is unclear. To fill this gap, we collected phenotypic and genetic data in up to 331 175 individuals from diverse ethnic groups. This

process involved a systematic review of the literature in PubMed, Web of Science, Embase and the NIH GWAS catalog complemented by data extraction from pre-existing GWAS or custom-arrays in consortia and single studies. We employed recently developed global meta-analytic random-effects methods to calculate summary odds ratios (OR) and 95% confidence intervals (CIs) or beta estimates and standard errors (SE) for the obesity status and BMI analyses, respectively. Significant associations were found with binary obesity status for rs6232 (OR = 1.15, 95% CI 1.06–1.24, $P = 6.08 \times 10^{-6}$) and rs6234/rs6235 (OR = 1.07, 95% CI 1.04–1.10, $P = 3.00 \times 10^{-7}$). Similarly, significant associations were found with continuous BMI for rs6232 ($\beta = 0.03$, 95% CI 0.00–0.07; $P = 0.047$) and rs6234/rs6235 ($\beta = 0.02$, 95% CI 0.00–0.03; $P = 5.57 \times 10^{-4}$). Ethnicity, age and study ascertainment significantly modulated the association of *PCSK1* polymorphisms with obesity. In summary, we demonstrate evidence that common gene variation in *PCSK1* contributes to BMI variation and susceptibility to common obesity in the largest known meta-analysis published to date in genetic epidemiology.

Introduction

The prevalence of obesity has reached epidemic proportions throughout the world (1). In addition to being the main risk predictor for the rapid increase of type 2 diabetes (T2D) (2), obesity also significantly increases the global disease burden of cardiovascular disease and cancer (3,4). Rising rates of childhood obesity, combined with an increasing prevalence of obesity in aging adult populations, suggest that the impact of this disease on human health will continue to grow in the future (5). Therefore, there is an urgent need to improve understanding of the etiology of obesity to help curb the obesity epidemic (6).

Genetic factors have been shown to play a substantial role in the etiology of obesity (7) and accordingly research has focused on identifying specific underlying genetic determinants of body weight regulation. Candidate-gene, gene-centric and genome-wide association (GWAS) studies have identified 42 loci with single-nucleotide polymorphisms (SNPs) that significantly associate ($P < 5 \times 10^{-8}$) with body mass index (BMI, as a continuous variable) (8–17). Additionally, case–control candidate gene and GWAS approaches have been used to examine the genetics of childhood and adult obesity (as a binary variable) (13,18–28). These studies have identified 46 loci with alleles associated with obesity at the genome-wide significance level. The majority of alleles ($N = 24$) influence both BMI variation and the risk for obesity, but 18 and 22 loci have been shown to contribute more specifically to BMI variation and the genetic risk for obesity, respectively (13,18–27). These data indicate that the genetic architecture of BMI variation and obesity may not be totally overlapping. Obesity may not only represents the extreme of the phenotypic spectrum of BMI (18), but perhaps a partially distinct inherited condition (29).

The *PCSK1* gene may be illustrative of this paradigm. Mutations in *PCSK1* lead to PC1/3 enzyme deficiency in neuroendocrine cells, which is characterized by monogenic obesity in mice and humans resulting from the abnormal maturation of hormones involved in energy and glucose metabolism (30–32). In a positional candidate-gene study, Benzinou *et al.* showed convincing evidence for the association of coding variants rs6232 and rs6234/rs6235 (pooled given perfect linkage disequilibrium between the two SNPs among diverse ethnic backgrounds) with childhood and adult severe obesity (27). The candidacy of these common variants is further strengthened in that they have been shown to reduce PC1/3 enzymatic activity through altered protein secretion, biosynthesis and catalytic activity (27,30,33,34) and determine glucose-stimulated proinsulin conversion (35,36), which is also a characteristic of complete human *PCSK1* deficiency (31).

However, replication of the association of *PCSK1* variants with obesity has provided conflicting results (27,37–45) with the lack of statistical power and genetic/phenotypic/ethnic heterogeneity being likely contributors to this variability. Additionally, conflicting evidence for the association of *PCSK1* variants with BMI variation has been observed in individual studies (35,37–40,43,44,46,47) and only nominal evidence of association with BMI variation has been found for rs6232 and rs6235 in large GWAS meta-analyses (12,36). To give a more conclusive answer regarding whether *PCSK1* variants differ in their contribution to extreme obesity (e.g. BMI ≥ 40 kg/m^2), common obesity (BMI ≥ 30 kg/m^2) and continuous BMI variation, we have systematically collected data from the literature and unpublished sources to perform a meta-analysis of the association of variants rs6232 and rs6234/rs6235 with quantitative BMI variation and common obesity risk in up to 331 175 subjects from diverse ethnic groups.

Results

Study selection

Results of the systematic search and data collection are presented in Figure 1. In total, 85 unique records were screened by title and abstract and 40 records were reviewed in full text, of which 10 were excluded. Reasons for exclusion after full text review included overlap with larger studies from the literature review, family-based studies (where clustering was not accounted for in the analysis), candidate-gene studies not examining the variants of interest, neither obesity nor BMI variation having been evaluated, lack of response from the authors and overlap with larger datasets. In total, 30 records were included from the literature, consortia, the Database of Genotypes and Phenotypes (dbGap), direct collaboration and novel contributions by the authors, as detailed in Supplementary Material, Table S1. Of these, 19 contained data on rs6232 and 28 contained data on rs6234/rs6235. Details and characteristics of all data sources included in the analysis can be found in Supplementary Material, Table S1. The minor allele frequency for each variant for rs6232, rs6234 and rs6235 are provided for the EpiDream cohort and from the 1000 Genomes Project in Supplementary Material, Tables S2 and S3, respectively.

Study quality

Study characteristics, genotyping and analysis methods of the included studies are described in the Supplementary Material, Table S1. As many studies were not initially designed to evaluate obese cases compared with non-obese controls, population structure was variable with many studies containing few obese cases and therefore likely underpowered to evaluate genetic associations with obesity. Hardy–Weinberg equilibrium was either reported or obtained via correspondence with $P > 0.05$ for all studies included. Similarly, all studies included were found to have SNP-wise call rates of >95% and all study estimates were adjusted for age and/or sex as covariates.

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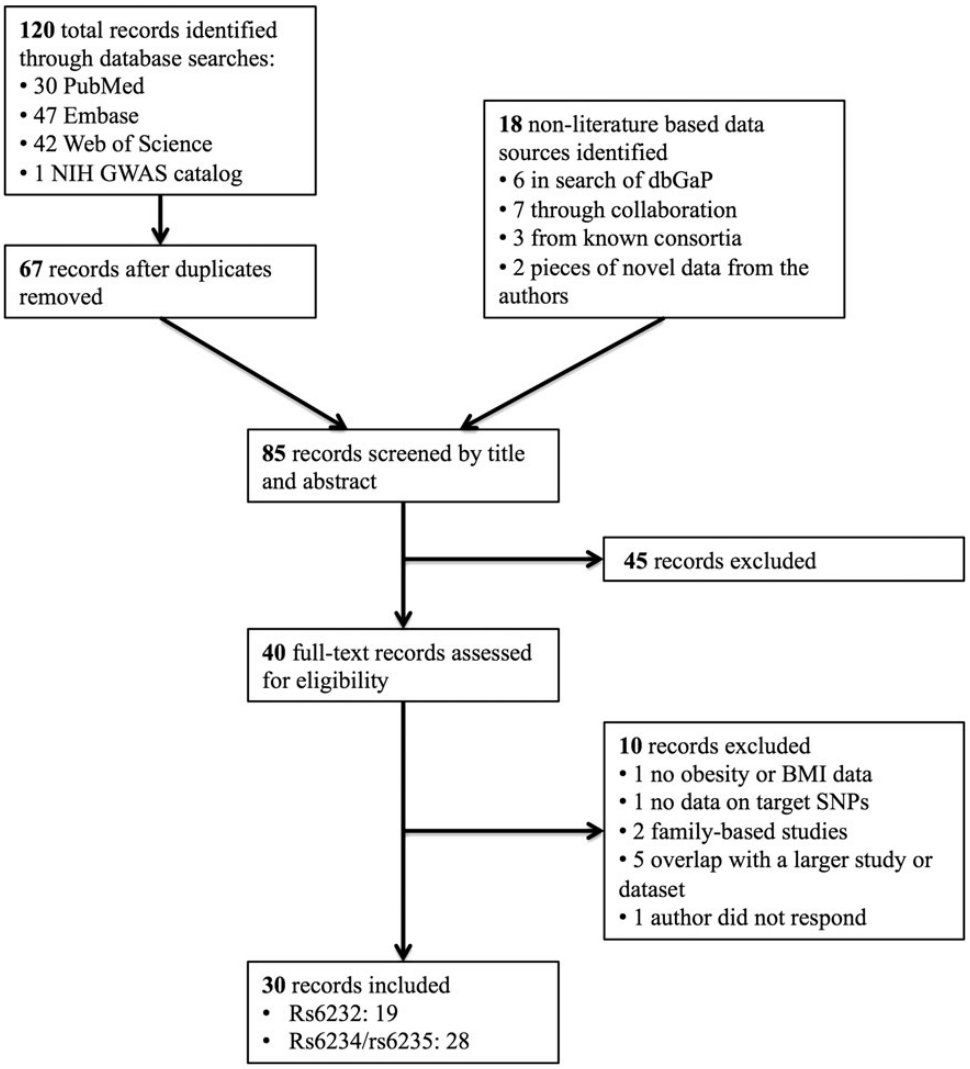

Figure 1. Flow diagram of literature search and study selection for meta-analysis of the association of rs6232 and rs6234/rs6235 with obesity and BMI. GWAS, genome-wide association study; dbGaP, Database of Genotypes and Phenotypes; BMI, body mass index; SNPs, single-nucleotide polymorphisms.

Binary obesity analysis

The results for the meta-analyses of rs6232 and rs6234/rs6235 with obesity are presented in Figure 2 using both classical random-effects meta-analytic techniques and a global random-effects meta-analytic method, designed to detect genetic associations among multi-cohort studies with high heterogeneity (48). The combined analysis of 131 284 individuals demonstrated a significant association of the G allele of rs6232 with obesity status [odds ratio (OR) = 1.15; 95% confidence interval (CI), 1.06–1.24] with a global method *P*-value (P_G) = 6.08×10^{-6} and a classical random-effects method *P*-value (P_C) = 4.38×10^{-4}. Similarly, the analysis of 239 581 individuals demonstrated an OR of 1.07 (95% CI 1.04–1.10; $P_G = 3.00 \times 10^{-7}$; $P_C = 2.75 \times 10^{-5}$) for the association of rs6234/rs6235 with obesity status. Low-to-moderate between-study heterogeneity was observed for the association of both rs6232 (I^2 = 43%; 95% CI, 9–64%, P = 0.011) and rs6234/rs6235 (I^2 = 68%; 95% CI, 54–77%, $P = 2.18 \times 10^{-9}$) with binary obesity. Finally, exclusion of the initial *PCSK1* discovery cohort (27) consisting of 1045 obese French adults and 1265 non-obese controls did not significantly impact the significance of our analysis for rs6232 (OR = 1.13; 95% CI 1.05–1.22; $P_G = 8.31 \times 10^{-5}$; P_C = 0.002) or rs6234/rs6235 (OR = 1.06; 95% CI 1.03–1.09; $P_G = 4.45 \times 10^{-5}$; $P_C = 2.05 \times 10^{-4}$).

Continuous BMI variation analysis

The results for the meta-analysis of rs6232 and rs6234/rs6235 with BMI variation are presented in Figure 3. In the analysis of 202 803 individuals, the average BMI increase for each G allele at rs6232 was 0.03 (95% CI 0.00–0.07; P_G = 0.047; P_C = 0.123). In the analysis of rs6234/rs6235, each effect-allele conferred a 0.02 (95% CI 0.00–0.03; $P_G = 5.57 \times 10^{-4}$; P_C = 0.008) unit increase in BMI among 331 175 individuals. The between-study heterogeneity was non-significant for the associations of rs6232 (I^2 = 30%; 95% CI, 0–59%, P = 0.102) and rs6234/rs6235 (I^2 = 22%; 95% CI, 0–50%, P = 0.137).

Heterogeneity and subgroup analysis

As low-to-moderate between-study heterogeneity was observed for the association of both rs6232 and rs6234/rs6235 with binary obesity, causes of heterogeneity were explored through pre-specified subgroup analyses (Table 1). Stratification by ethnicity,

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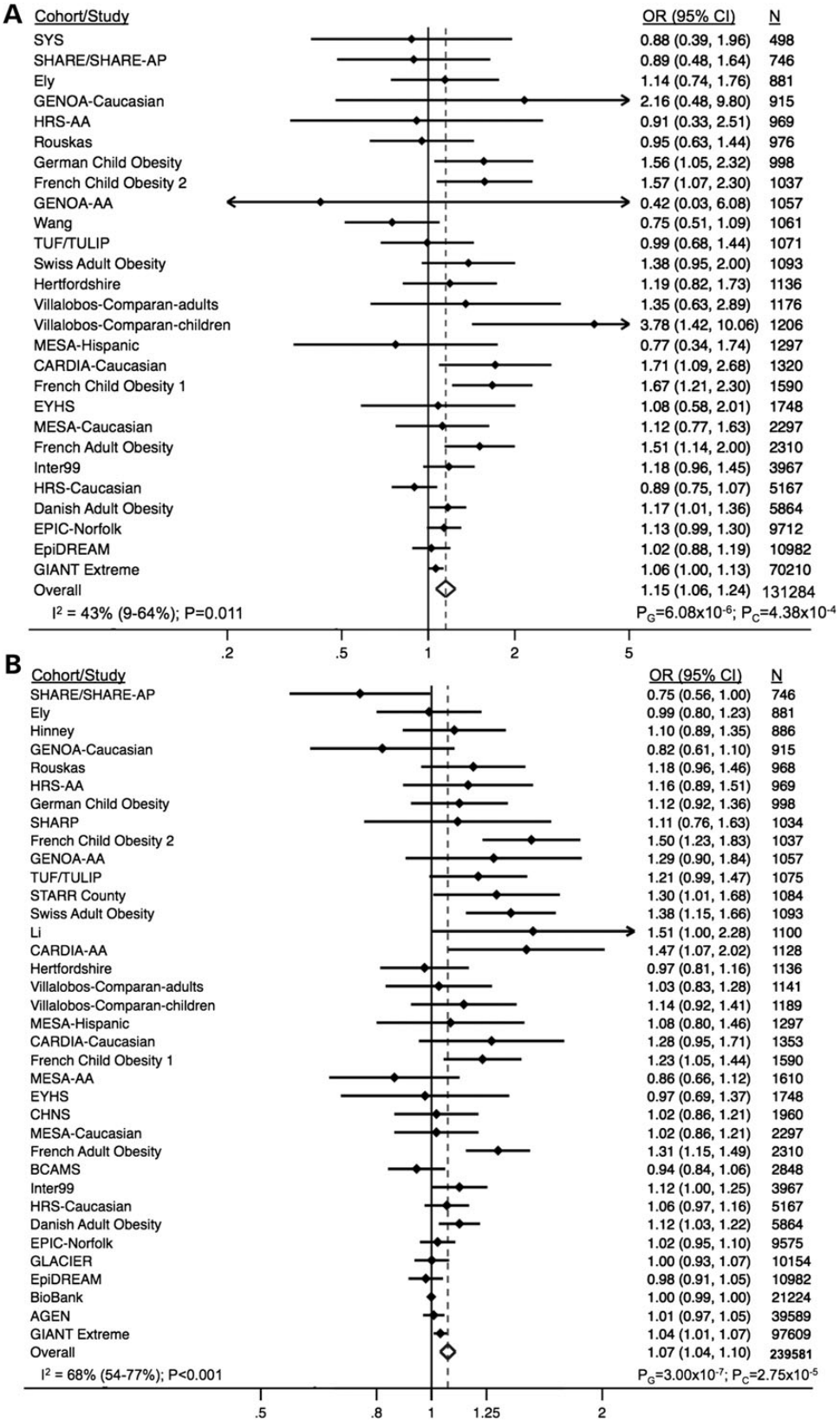

Figure 2. Association of rs6232 (**A**) and rs6234/rs6235 (**B**) with obesity status. OR, odds ratio; CI, confidence interval.

cohort size (≤1000 or >1000) and study ascertainment did not have any significant impact on the association of rs6232 with obesity. Stratification by cohort age-group (child/adolescent versus adult) resulted in a significantly different association between rs6232 and obesity in children/adolescents (OR = 1.53, 95% CI 1.22–1.93; $P_G = 3.68 \times 10^{-5}$; $P_C = 2.84 \times 10^{-4}$) and in adults

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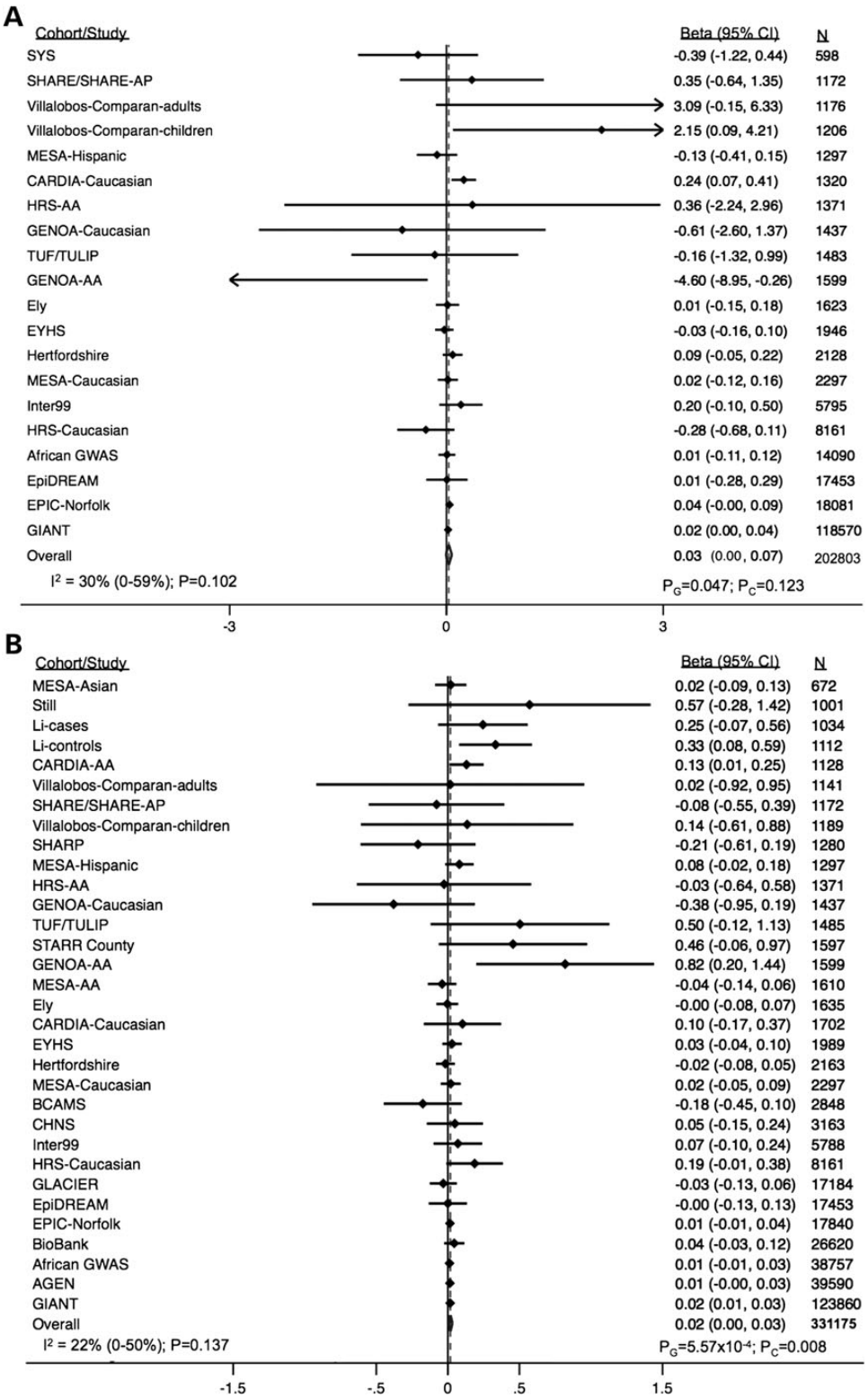

Figure 3. Association of rs6232 (**A**) and rs6234/rs6235 (**B**) with BMI. OR, odds ratio; CI, confidence interval.

(OR = 1.10, 95% CI 1.03–1.17; $P_G = 0.001$; $P_C = 0.006$; $P_{difference} = 3.00 \times 10^{-4}$). Between-study heterogeneity still remained significant for the association between rs6232 and obesity, after stratifying by ethnicity, cohort size or study ascertainment (Table 1). However, no more between-study heterogeneity was observed when children/adolescent and adult subgroups were analyzed apart ($P = 0.250$ and 0.182, respectively).

Stratification by cohort size also did not have a significant impact on the association of rs6234/rs6235 with obesity. However, stratification by ethnicity, cohort age-group (child/adolescent

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Table 1. Subgroup analysis for the association of rs6232 and rs6234/rs6235 with obesity

rs6232	Random-effects OR (95% CI)	P-value Classical	Global	Heterogeneity I^2 (95% CI)	P-value	χ^2-test for difference	No. of studies/ cohorts	Sample size
Ethnicity								
White Caucasian	1.14 (1.06–1.24)	0.001	1.08×10^{-5}	44 (7–66)	0.015	0.485	22	125 579
East Asian							0	0
Hispanic	1.52 (0.65–3.55)	0.335	0.182	67 (0–90)	0.049		3	3679
African	0.83 (0.32–2.13)	0.694	0.810	[a]	0.597		2	2026
Cohort age-group								
Child/adolescent	1.53 (1.22–1.93)	2.84×10^{-4}	3.68×10^{-5}	25 (0–68)	0.250	3.00×10^{-4}	6	7077
Adult	1.10 (1.03–1.17)	0.006	0.001	22 (0–54)	0.182		21	124 207
Cohort size								
≤1000	1.14 (0.92–1.40)	0.223	0.351	0 (0–71)	0.536	0.756	7	5983
>1000	1.16 (1.06–1.26)	0.003	1.17×10^{-5}	53 (21–72)	0.003		20	125 301
Population-based recruitment								
No	1.19 (1.02–1.38)	0.030	7.15×10^{-4}	53 (13–75)	0.012	0.222	13	29 799
Yes	1.12 (1.03–1.22)	0.010	0.004	28 (0–62)	0.157		14	101 485
rs6234/rs6235								
Ethnicity								
White Caucasian	1.09 (1.04–1.14)	2.04×10^{-4}	4.28×10^{-9}	64 (44–77)	1.25×10^{-5}	5.13×10^{-7}	23	163 385
East Asian	1.00 (0.98–1.03)	0.961	1.00	22 (0–67)	0.274		5	66 721
Hispanic	1.13 (0.99–1.27)	0.050	0.103	0 (0–85)	0.579		4	4711
African	1.16 (0.92–1.46)	0.222	0.115	59 (0–86)	0.064		4	4764
Cohort age-group								
Child/adolescent	1.13 (1.00–1.29)	0.053	5.61×10^{-4}	68 (29–86)	0.004	0.005	7	10 296
Adult	1.06 (1.02–1.09)	0.001	6.35×10^{-5}	66 (49–77)	4.19×10^{-7}		29	229 285
Cohort size								
≤1000	1.02 (0.91–1.15)	0.69	0.576	44 (0–77)	0.096	0.439	7	6363
>1000	1.07 (1.04–1.11)	2.56×10^{-5}	3.48×10^{-7}	71 (58–80)	1.71×10^{-9}		29	23 3218
Population-based recruitment								
No	1.11 (1.05–1.17)	1.60×10^{-4}	3.65×10^{-6}	76 (61–85)	1.35×10^{-7}	0.001	16	92 067
Yes	1.05 (1.00–1.09)	0.040	0.005	46 (8–68)	0.014		20	14 7514

OR, odds ratio; CI, confidence interval.
[a]Analysis not possible if <2 degrees of freedom.

versus adult) or study ascertainment resulted in a significantly different association between rs6234/rs6235 and obesity (Table 1). The OR estimate of rs6234/rs6235 for obesity was comparable in white Caucasian (OR = 1.09, 95% CI 1.04–1.14; $P_G = 4.28 \times 10^{-9}$; $P_C = 2.04 \times 10^{-4}$), Hispanic (OR = 1.13, 95% CI 0.99–1.27; $P_G = 0.103$; $P_C = 0.050$) and African (OR = 1.16, 95% CI 0.92–1.46; $P_G = 0.115$; $P_C = 0.222$) ethnic groups, but the rs6234/rs6235 variant conferred no evidence for an increase in the odds for obesity in East Asian populations (OR = 1.00, 95% CI 0.98–1.03; $P_G = 1.00$; $P_C = 0.961$; $P_{difference} = 5.13 \times 10^{-7}$), which included Chinese, Japanese, Korean and Malay individuals. A significantly different association between rs6234/rs6235 and obesity was observed in children/adolescents (OR = 1.13, 95% CI 1.00–1.29; $P_G = 5.61 \times 10^{-4}$; $P_C = 0.053$) and in adults (OR = 1.06, 95% CI 1.02–1.09; $P_G = 6.35 \times 10^{-5}$; $P_C = 0.001$; $P_{difference} = 0.005$). The association of rs6234/rs6235 with obesity was significantly different depending on the type of recruitment [population-based or other recruitment (e.g. hospital): OR = 1.05, 95% CI 1.00–1.09; $P_G = 0.005$; $P_C = 0.040$ and OR = 1.11, 95% CI 1.05–1.17; $P_G = 3.65 \times 10^{-6}$; $P_C = 1.60 \times 10^{-4}$, respectively; $P_{difference} = 0.001$]. Between-study heterogeneity still remained significant for the association between rs6234/rs6235 and obesity, after stratifying by ethnicity, cohort age-group, cohort size or study ascertainment (Table 1).

There was no evidence of small study effects in the analysis of rs6232 for obesity (Supplementary Material, Figure S1). However, a funnel plot for rs6234/rs6235 demonstrated asymmetry for the obesity analysis (Supplementary Material, Figure S2), which was supported by the Egger's ($P < 0.05$), but not the Begg's ($P > 0.05$) tests. Additionally, the association of rs6234/rs6235 with obesity status did not significantly differ in studies with greater than 1000 individuals compared with those with 1000 participants or less ($P = 0.439$).

Table 2. Association of rs6232 and rs6235 with obesity class in GIANT

	OR (95% CI)	P-value	Cases	Controls
rs6232				
Overweight	1.04 (1.00–1.08)	0.078	78 671	60 578
Obesity class I	1.06 (1.00–1.13)	0.057	22 947	47 263
Obesity class II	1.08 (0.98–1.20)	0.120	5983	35 721
Obesity class III	1.13 (0.95–1.34)	0.180	1534	23 221
rs6235				
Overweight	1.03 (1.01–1.05)	0.005	92 808	65 660
Obesity class I	1.04 (1.01–1.07)	0.010	32 746	64 864
Obesity class II	1.07 (1.02–1.11)	0.003	9723	61 085
Obesity class III	1.11 (1.02–1.20)	0.011	2550	35 900

OR, odds ratio; CI, confidence interval.

Finally, published data from the Genetic Investigation of Anthropometric Traits (GIANT) consortium for the association of rs6232 and rs6235 with different classes of obesity are presented in Table 2. In all analyses, the control group consisted of normal weight individuals (BMI < 25 kg/m^2). For the rs6232 variant, there

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was an OR of 1.04 (95% CI 1.00–1.08), 1.06 (95% CI 1.00–1.13), 1.08 (95% CI 0.98–1.20) and 1.13 (95% CI 0.95–1.34), across the overweight and obese classes I, II and III groups, respectively ($0.057 \leq P_C \leq 0.18$). Similarly, the rs6235 analysis showed a similar increasing risk pattern with an OR of 1.03 (95% CI 1.01–1.05), 1.04 (95% CI 1.01–1.07), 1.07 (95% CI 1.02–1.11) and 1.11 (95% CI 1.02–1.20), across the overweight and obese classes I, II and III groups, respectively ($0.003 \leq P_C \leq 0.011$). However, the ORs of rs6232 and rs6235 SNPs for different classes of obesity were not significantly different ($P > 0.05$).

Discussion

In this study, we provide evidence that common variants in *PCSK1* contribute to BMI variation and common obesity. The proprotein convertase 1 encoded by the *PCSK1* gene belongs to the subtilisin-like proprotein convertase family. Proprotein convertase 1 is known to cleave key peptides in the regulation of energy balance such as proinsulin or proopiomelanocortin (49). Rare loss-of-function coding mutations in *PCSK1* have been associated with a Mendelian form of hyperphagic obesity in mice and humans (30,32,50). Benzinou and colleagues provided evidence of association between the frequent coding variants N221D (rs6232) and Q665E/S690T (rs6234/rs6235) and childhood and adult severe obesity in European populations (27). However, conflicting results have been reported regarding the association of the rs6232 and rs6234/rs6235 polymorphisms with common obesity (27,37–45) or BMI variation (12,35–40,43,44,46,47) in diverse ethnic backgrounds. This prompted us to assess the contribution of rs6232 and rs6234/rs6235 with common obesity and BMI variation using a meta-analytic approach. We enhanced the power of our analyses, and therefore the ability to detect associations, using both a classic meta-analytic approach in addition to data extraction from pre-existing GWAS or custom-arrays from consortia and single studies as well as internal data and collaboration. In total, we collected phenotypic and genetic data in up to 331 175 individuals from diverse ethnic groups, which represents to our knowledge the largest meta-analysis published to date in the field of genetic epidemiology.

Overall, our data demonstrate that the common functional variants rs6232 and rs6234/rs6235 in *PCSK1* not only predispose to severe obesity but also modestly increase the risk of common obesity and increased BMI within populations. Our results are in line with the conclusions of a large-scale GWAS meta-analysis for BMI and different clinical classes of obesity performed in up to 263 407 subjects (20). In this study, Berndt *et al.* found a large overlap of SNPs contributing to both quantitative BMI and different thresholds of obesity and concluded that there was little etiological heterogeneity between these traits at least at the level of common SNP variation (20). Our data are only partially concordant with a recent meta-analysis published by Stijnen and colleagues (51). However, the 65% lower sample size in this study in comparison with ours ($N = 200\,000$ versus 331 175), added to methodological considerations lead us to interpret the conclusions of this study with caution (52).

Children with congenital proprotein convertase one-third deficiency display early growth abnormalities and reduced height as a consequence of growth hormone deficiency (53). Therefore, we cannot totally exclude that the association of rs6232 and rs6234/rs6235 SNPs with BMI may be in part confounded by an additional genetic effect on height variation. We consulted the publically available data released in 2014 by the GIANT consortium (54) and did not find an association between the rs6232 SNP and height ($B = -0.0086 \pm 0.0068$, $P = 0.21$, $N = 233\,697$). On the contrary, and in line with the observations made in monogenic patients, the rs6235 SNP BMI-increasing allele evidenced a strong association with decreased height ($B = -0.0224 \pm 0.0033$, $P = 5.4 \times 10^{-11}$, $N = 251\,342$).

If the genetic architecture of BMI variation in general populations and the risk of common obesity includes many overlapping genetic variants, these variants may be more prevalent in severe and/or familial forms of childhood and adult obesity. Benzinou *et al.* indeed reported odds ratios (OR) for obesity of 1.34 (95% CI 1.20–1.49; $P = 7.27 \times 10^{-8}$) and 1.22 (95% CI 1.15–1.29; $P = 2.31 \times 10^{-12}$) for rs6232 and rs6234/rs6235 in a European sample enriched in familial forms of childhood and adult extreme obesity in European populations (27). Lower OR for common obesity were observed for rs6232 (OR = 1.15) and rs6234/rs6235 (OR = 1.07) in our meta-analysis. Importantly, the larger ORs for obesity observed in the Benzinou *et al.* original study are less likely to result from initial overestimation of the true effect (i.e. winner's curse), as they were estimated from a meta-analysis of seven independent cohorts (27). Additionally, exclusion of the initial *PCSK1* discovery cohort in sensitivity analyses did not impact the significance of our results. A more plausible explanation is that extreme familial forms of early-onset and adult obesity are enriched for susceptibility variants. Consistent with this hypothesis, a non-significant progressive enrichment of rs6232 and rs6234/rs6235 effect variants was observed for increasing degrees of obesity in the GIANT sample (Supplementary Material, Table S3). Our results indicate that an enrichment sampling strategy (selection of obese individuals having a strong family background of the disease, an early age of onset and/or a more severe phenotype) is a cost-effective and efficient approach to identify loci that also contribute to BMI variation and risk for common obesity in general populations (20,55).

Our data show a substantial degree of between-study heterogeneity in the association of *PCSK1* SNPs rs6232 and rs6234/rs6235 with obesity. Benzinou *et al.* similarly reported significant between-study heterogeneity while analyzing the association of the SNP rs6235 with severe obesity in seven independent cohorts (27). We investigated the potential causes of this heterogeneity and made several observations. Ethnicity significantly modulated the association between rs6234/rs6235 and obesity. Whereas similar effect sizes for the association of rs6234/rs6235 with obesity were found in white Caucasian, African and Hispanic ethnic groups, no evidence for association was found in East Asian populations. As the rs6234/rs6235 coding non-synonymous SNPs exhibit functional effects on the proprotein convertase 1 activity (34), and therefore do not represent proxy SNPs, the absence of evidence for an association restricted to only East Asian populations is very unlikely to be explained by differential linkage disequilibrium structure at the *PCSK1* locus in certain ethnic groups. Notably, another SNP in *PCSK1*, independent of the rs6234/rs6235 signal, has been recently identified as an important contributor to BMI variation in East Asian populations (15). Therefore, the lack of association of rs6234/rs6235 with obesity in East Asians in the current study is unlikely to be explained by an interaction between the *PCSK1* locus and ethnic-specific lifestyle factors that may inhibit the genetic effect on obesity at this specific locus. Ethnic-specific epistasis effects may account for this intriguing pattern of association. In contrast to rs6234/rs6235, ethnicity did not modulate the association between rs6232 and obesity. However, this may have been secondary to decreased power and no available data examining the association of rs6232 and obesity or BMI in East Asian populations.

Study ascertainment in the overall meta-analysis significantly modulated the association of rs6234/rs6235 with obesity. Not surprisingly, obese cases recruited in hospitals, which tended to be

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more obese, or obtained from pedigrees enriched in obese cases displayed 2-fold higher ORs for obesity in comparison with cases issued from the general population. Additionally, there is a suggestion of possible small study effects for the rs6234/rs6235, due to a lower than predicted representation of smaller negative studies in our analysis, which may also explain a degree of the observed heterogeneity.

Cohort age-group significantly modulated the association between rs6232, rs6234/rs6235 and obesity with the effect sizes for both SNPs being stronger in children/adolescents than in adults (Table 1). Similar trends have been observed for the association of rs6232 with BMI and obesity in younger *versus* older adult Europeans in two independent reports (38,44). As prohormone convertase 1 cleaves proopiomelanocortin, a key peptide in the regulation of energy balance and appetite, it is tempting to speculate that the genetic effect of common functional variants in *PCSK1* may be more pronounced in the context of the more recent 'obesogenic' environment with unlimited access to high-caloric food (56).

Additional environmental or biological factors (e.g. physical activity) are likely to modulate the association between rs6234/rs6235 and obesity in the current study. Between-study heterogeneity indeed remained significant after stratifying the genetic association test by ethnicity, cohort age-group or population recruitment. Our data, in line with previous reports, confirm that heterogeneity may be a common feature of genetic association studies involving obesity predisposing variants (57–59). In that context, the global meta-analytic random-effects method recently developed by Lebrec *et al.* is especially relevant as it is designed to detect genetic associations among multi-cohort studies that convey a high level of between-study heterogeneity (48). Neupane and colleagues recently demonstrated that the global method achieves higher power and lower rates of false positives compared with classic methods in the presence of high between-study heterogeneity, using both simulated and real datasets (60). We observed the same trends in our study with the global method providing greater precision of estimates than the classic random-effects inverse variance weighted method in the setting of high between-study heterogeneity. We therefore support the wider use of global meta-analytic random-effects model for the genetic dissection of complex traits.

The strengths of this meta-analysis include a comprehensive data collection strategy, an exceptionally large sample size representative of high ethnic diversity, the use of the most up-to-date meta-analytic methods and the selection of functionally relevant coding polymorphisms. Limitations of this study include the modest statistical power of subgroup analyses and the limited access to a broad range of environmental exposure information that may modulate the association of *PCSK1* SNPs with obesity.

In summary, we provide evidence that SNPs rs6232 and rs6234/rs6235 in *PCSK1* contribute to BMI variation as well as increased susceptibility to common obesity. Our data confirm the power of gene identification strategies based on extreme forms of obesity to identify loci that also contribute to BMI variation and risk for common obesity in general populations.

Methods

The PRISMA statement guidelines were utilized for this systematic review and meta-analyses (61).

Eligibility criteria

Individual studies, meta-analyses and GWAS consortia examining obesity status or BMI variation with respect to exposure to the previously defined effect alleles of rs6232 (G), rs6234 (G) or rs6235 (C) were eligible for inclusion (27,41). As rs6234 and rs6235 are in perfect linkage disequilibrium among diverse ethnic backgrounds ($r^2 = 1.0$), they are considered as identical and are analyzed together (62,63). Additionally, studies in East Asian, South Asian and white Caucasian populations reporting on the SNP rs7713317 (effect allele: G) were eligible, as this variant is in perfect linkage disequilibrium ($r^2 = 1.0$) with rs6234 and rs6235 among these groups in both the HapMap (62) and 1000 Genomes projects (63). Only analyses of variants under the additive model were eligible for inclusion as this has been demonstrated to be the most likely inheritance model (27). In addition, studies that recorded BMI or obesity status and used genotyping platforms known to contain these variants were also eligible and corresponding authors were contacted to share the unpublished data in a collaborative manner. Studies examining obesity status were eligible if they compared an obese group with a non-obese group as defined according to the study population. Our exclusion criteria were clustered datasets such as family-based studies (unless clustering was accounted for in the analysis), data from the analysis of variants not shown to be in Hardy–Weinberg equilibrium of $P \geq 0.05$ and data with an imputation quality of <0.9.

Information sources

The search strategy was designed to identify all sources of published and unpublished data both in the literature and in available databases. Electronic searches without language or date restriction were carried out in PubMed (1966–present), Web of Science (1899–present), Embase (1974–present) and the NIH GWAS catalog (64). Search terms used in all databases to identify relevant studies, along with a representative search strategy used for the PubMed query, can be found in Supplementary Material, Table S4. All articles identified through the search were evaluated based on the title and abstract. Clearly, irrelevant studies were excluded from further consideration. The remaining articles received a full text review. The last search was undertaken on 9 June 2013.

Studies recording data on study participant BMI and/or obesity status and utilizing genotyping arrays known to include rs6232, rs6234, rs6235 or rs7713317 were searched for in dbGaP (65). Relevant genotyping arrays and proxy SNPs were identified using the SNP Annotation and Proxy Search (66). Additional data were obtained through collaboration with other investigators and through consortia (Supplementary Material, Table S1).

Descriptive information was extracted from each study including: (i) SNPs available, (ii) study design, (iii) participant selection, (iv) eligible phenotype (obesity or BMI), (v) genotyping method, (vi) sample size, (vii) number of obese and non-obese individuals (for obesity analyses), (viii) obese and non-obese definitions, (ix) ethnicity, (x) included age groups and (xi) model adjustments. As data were gathered from individual studies, meta-analyses and GWAS consortia, particular care was taken to avoid the inclusion of duplicate data. Detailed cohort information was requested from participating consortia and key study characteristics were compared across all eligible studies to determine if multiple publications with data from the same study were present. If study duplication existed, data from the publication with the most complete analysis of the duplicated study was used. Where possible, corresponding authors were contacted to provide study data that did not overlap with other data sources. The literature search and article review were carried out independently by two reviewers (K.N. and D.M.) with consensus reached by discussion. Details of study selection can be found in Figure 1.

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Unpublished data from the EpiDREAM (Epidemiological arm of the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) (67), SHARE (Study of Health Assessment and Risk in Ethnic groups) (68) and SHARE-AP (Study of Health Assessment and Risk Evaluation in Aboriginal Peoples) (69) cohorts were also included. Briefly, the EpiDREAM multi-ethnic longitudinal cohort is the epidemiological arm of the DREAM study comprised of individuals with an increased risk for T2D who were screened for trial eligibility. The SHARE and SHARE-AP studies are cross-sectional cohorts investigating atherosclerosis and cardiovascular diseases among different ethnic groups living in Canada. Genetic and clinical data were available from 1172 participants from the SHARE and SHARE-AP population. In the EpiDREAM study, 17 453 subjects from six ethnic groups (South Asian, East Asian, European, African, Latin American and Native North American) and having both genetic and baseline clinical information have been included here. Self-reported ethnicity has been validated in the SHARE, SHARE-AP and EpiDREAM studies using the eigensoft software (http://genepath.med.harvard.edu/~reich/Software.htm). The SHARE, SHARE-AP and EpiDREAM cohorts were genotyped using the cardiovascular gene-centric 50 K SNP ITMAT-Broad-CARe (IBC) array (70). Subjects in the SHARE and SHARE-AP cohorts were genotyped using the Illumina HumanCVD BeadChip. SNPs rs6232, rs6234 and rs6235 in *PCSK1* were part of the 50K array SNP list and were extracted for further study.

Statistical analysis

The primary analyses examined the effect of exposure to the effect alleles of rs6232 (G) or rs6234/rs6235/ rs7713317 (G/C/C) on obesity status and BMI variation. The OR and 95% CI were collected to examine the effects of the variants on obesity status. The beta estimates and SE were collected to examine the effects of the variants on BMI. If eligible studies did not report data necessary for inclusion in the meta-analysis, the corresponding authors were contacted directly.

We implemented a global random-effects meta-analytic method, designed to detect genetic associations among multicohort studies with high heterogeneity (48), to calculate a summary OR and 95% CI or summary beta estimate and SE for the obesity status and BMI variation analyses, respectively. Compared with classical random-effects meta-analytic techniques, which consider each study to be a random sample of the true effect distribution and calculates the combined effect estimate as the mean of this distribution, the global random-effects method tests if the overall association or between-cohort variance of associations is non-zero. This approach was implemented because it has demonstrated improved power and lower rates of false positives compared with classical methods in the setting of heterogeneity (60). The meta-analyses were additionally performed using classical random-effects inverse variance weighted methods.

Between-study heterogeneity was evaluated using Cochran's Q-statistic and the proportion of heterogeneity due to study variation was quantified using the I^2 statistic (71). If substantial heterogeneity was detected ($P < 0.1$), sources of this heterogeneity were explored. The presence of small study effects was evaluated using funnel plots, by calculating Begg and Egger statistics and by comparing subgroup analysis of small studies ($n \leq 1000$) versus large studies ($n > 1000$) as previously described (72). Subgroup analysis was further examined according to pre-specified categories including ethnicity (white Caucasian, East Asian and Hispanic, African), studies using population-based recruitment compared with studies using other recruitment methods (e.g. hospital based) and by age category. Within subgroup heterogeneity was examined using a chi-squared test for difference.

Additionally, using summary statistics from the GIANT consortium (20), we examined the risk of obesity conferred by the *PCSK1* variants with increasing classes of obesity including overweight (BMI $\geq$ 25 kg/m^2) and obesity classes I (BMI $\geq$ 30 kg/m^2), II (BMI $\geq$ 35 kg/m^2) and III (BMI $\geq$ 40 kg/m^2) compared with a lean control group (BMI < 25 kg/m^2).

All analyses were carried out using Stata version 12 (StataCorp, College Station, TX, USA) or the R software.

Supplementary Material

Supplementary Material is available at *HMG* online.

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