ELECTRONIC LETTER

Association of the 103I *MC4R* allele with decreased body mass in 7937 participants of two population based surveys

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Background: The melanocortin-4-receptor gene (*MC4R*) is part of the melanocortinergic pathway that controls energy homeostasis. In a recent meta-analysis, the *MC4R* V103I (rs2229616) polymorphism was shown to be associated with body weight regulation. Although no functional differences between the isoleucine comprising receptor and the wild type receptor have been detected as yet, this meta-analysis of 14 case–control studies reported a mild negative association with obesity (odds ratio (OR) 0.69, p = 0.03). However, evidence in a large population based study in a homogeneous population and a significant estimate of the change in quantitative measures of obesity is still lacking.

Methods: We analysed the data of two surveys of a white population with the same high quality study protocol, giving a total of 7937 participants.

Results: By linear regression, we found a significant decrease of 0.52 body mass index (BMI) units (95% confidence interval (CI) −0.02 to −1.03, p = 0.043) for carriers of the heterozygote rs2229616G/A genotype, which was observed in 3.7% of the participants. Logistic regression yielded a significantly negative association of the *MC4R* variant with ''above average weight'' (BMI ⩾ median BMI) yielding an OR of 0.75 (95% CI 0.59 to 0.95 p = 0.017). We obtained similar results comparing obese (BMI ⩾30 kg/m^2, World Health Organization results for 1997) with non-obese (BMI <30 kg/m^2) participants. The results were found for both sexes and each survey separately, and did not depend on the modelling of age, sex, or survey effects.

Conclusions: Our study confirms previous findings of a meta-analysis that the relatively infrequent G/A genotype of the V103I *MC4R* polymorphism is negatively associated with above average weight and obesity in population based original data of 7937 participants, and extends previous findings by showing for the first time a significantly lower BMI in individuals carrying the infrequent allele of this *MC4R* variant.

The melanocortin-4 receptor gene (*MC4R*) is part of the melanocortinergic pathway, which controls energy homeostasis, and it was recently suggested to be a major gene for obesity.[1 2] *MC4R* mutations have been detected in up to 6% of obese individuals.[2 3] The isoleucine allele of the V103I polymorphism (rs2229616; G/A) has been found in ~3% of individuals in white populations and is thus the most common among the *MC4R* variants.[2 4] Although pharmacological studies have not detected functional differences between the isoleucine comprising receptor and the wild type receptor,[1 4] a meta-analysis of 14 case–control studies reported a negative association of the 103 isoleucine allele with obesity (odds ratio (OR) 0.69, p = 0.03). Therefore, this variant or a variant in linkage disequilibrium or the respective haplotype were suggested to cause a moderate gain of function of MC4R.[4]

In the recent meta-analysis, the infrequent *MC4R* 103I allele was shown to be negatively associated with obesity.[4] The initial finding was based on a significantly reduced transmission of the 103I allele in 520 trios ascertained via an obese offspring. The subsequent meta-analysis, comprising 7713 individuals from 14 studies was performed using heterogeneous definitions of ''obesity'' and involved heterogeneous populations. Furthermore, subjects with intermediate BMI were disregarded in all but four studies, as only obese (defined by the 90th BMI percentile or by the cutoff points 28, 30, 35, or 40 kg/m^2 depending on the study) and non-obese subjects (defined by the 50th BMI percentile or the cutoff points 22, 25, 20, or 30 kg/m^2 depending on the study) were included. A change in BMI of 0.48 kg/m^2 by the rare *MC4R* variant, which lacked statistical significance (p = 0.22) was found by quantitative analysis based on one of the studies.[4] Although the meta-analysis was an important step in providing first evidence for an association of this *MC4R* variant with human obesity, more and higher quality data are required to validate the finding on a population basis.

It was the aim of our investigation not only to show a negative association between the *MC4R* 103I variant and obesity on a population basis, but also to specify the extent to which the variant is associated with decreased body mass. We therefore analysed the data from two large population based surveys from the Augsburg region of southern Germany: one survey for the years 1994–1995 (S3) and one for 1999–2001 (S4). In the present study, 7937 participants were thus analysed with respect to *MC4R* 103I, which, to our knowledge, is one of the largest population based genetic association studies on obesity performed to date.

MATERIAL AND METHODS

The study data

In the southern German region of Augsburg including the city of Augsburg and the two surrounding counties, population based surveys of the 25–74 year old population in groups of 5 year age range were implemented in 1984 as part of the WHO MONICA (multinational MONItoring of trends and determinants in CArdiovascular disease) project and continued since 1996 within the KORA (KAoperative Gesundheitsforschung in der Region Augsburg) platform. The aims of the surveys were to describe the prevalence of common diseases and their risk factors in a representative sample of the adult general population.[6]

The current study used the survey of the years 1994–1995 (MONICA, S3) comprising 4856 participants and the survey of the years 1999–2001 (KORA, S4) comprising 4261 participants, yielding 9117 recruited participants. The study

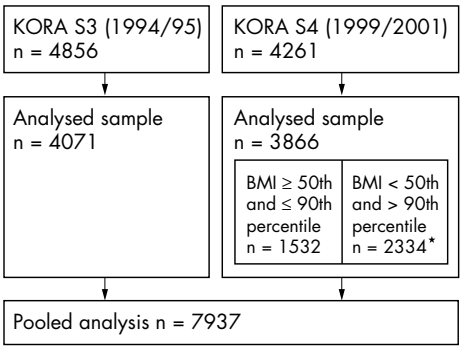

Figure 1 Overview of the composition of the pooled analysed data from the two surveys. *Included in meta-analysis by Geller *et al*.[4]

population of S3 and S4 comprised all German residents of the Augsburg region born between 1920 and 1975 identified through the public record office. More than 99.5% of the participants were white. The high standards of the WHO MONICA project applied to both surveys. All study participants underwent a standardised interview, physical examination, and blood withdrawal by trained staff. They gave informed written consent on a form of the Bavarian ethics committee and the ethics committee of the University of Munich.

For determination of body weight and height, participants were asked to remove shoes and heavy clothing. The weight measurements were performed with a calibrated body weight scale (SECA 709) and were carried out with an accuracy of 0.1 kg. The body height was read to the nearest 0.5 cm on a body height scale. BMI (kg/m^2) was calculated as weight in kilograms divided by height in square metres. All participants of S3 and S4 with available DNA and BMI were eligible and genotyped with respect to *MC4R* V103I. Genotyping was performed using a matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry system (Sequenom, Mass EXTEND, San Diego, USA) as described earlier.[4] The call rates were 99.3% and 97.9%, respectively, for S3 and S4.

The pooled data analysis included 7937 individuals with complete information on age, sex, BMI, and the *MC4R* genotype, 4071 from S3 and 3866 from S4. There was no overlap between the two surveys by design. From S4, individuals with a BMI <50th percentile or ⩾90th percentile (n = 2334; 60% of the data from that particular survey) had already been included in the aforementioned meta-analysis.[4] The composition and derivation of the analysed pooled sample is summarised in fig 1.

Statistical methods

Two different statistical approaches were applied to analyse this association in the 7937 participants representative of the southern German population. Firstly, using a linear regression model with BMI as continuous outcome for each study separately and for both studies combined, the unit change in BMI by the *MC4R* 103I variant was estimated. Secondly, by logistic regression analysis, we computed OR estimates using two different BMI cut off points: (*a*) individuals with BMI ⩾ the age, sex, and survey specific median BMI (''above average BMI'') compared with those with BMI below the median (the respective median BMI for each age, sex, and study stratum are shown in table 1); and (*b*) individuals with BMI ⩾30 kg/m^2 (''obese'' according to the WHO definition) compared with those with BMI <30 kg/m^2.

All analyses were adjusted for gender and 5 year age groups assuming a trend per group. Interaction effects of age and sex or the *MC4R* variant were explored. In the combined analysis, an effect of the study was additionally estimated; heterogeneity of the studies was tested by including interaction of study with any of the covariates in the model (*MC4R* polymorphism, age group, sex).

RESULTS

In the present investigation, two population based surveys from the Augsburg region with complete genotype and

Table 1 Subject characteristics in the analysed samples of the two surveys

	Survey S3 (1994/1995)		Survey S4 (1999–2001)	
	Men	Women	Men	Women
Number of subjects				
All	2019	2052	1919	1947
By BMI WHO category (kg/m^2)				
<18.5	6 (0.3%)	16 (0.8%)	4 (0.2%)	20 (1.0%)
18.5–20	537 (26.6%)	886 (43.2%)	494 (25.7%)	779 (40.0%)
25–30	1084 (53.7%)	693 (33.8%)	994 (51.8%)	676 (34.7%)
30–35	328 (16.2%)	320 (15.6%)	344(17.9%)	324 (16.6%)
35–40	54 (2.7%)	102 (5.0%)	60 (3.1%)	112 (5.7%)
40+	10 (0.5%)	35 (1.7%)	23 (1.2%)	36 (1.8%)
By obesity status				
BMI ⩾30	392 (19.4%)	457 (22.3%)	427 (22.2%)	472 (24.2%)
BMI <30	1627 (80.6%)	1595 (77.7%)	1492 (77.8%)	1475 (75.8%)
BMI (kg/m^2)				
Min–max	16–46	14–57	16–55	16–51
p50*, p90†	27/32	26/33	27/32	26/34
p50/p90 by age group				
25–30	24.5/28.0	22.4/27.6	24.8/29.3	22.8/30.0
30–35	25.8/31.1	23.0/30.0	25.5/30.4	23.8/31.6
35–40	26.2/31.9	24.0/31.9	26.4/30.9	24.4/31.0
40–45	26.6/31.7	24.0/31.9	26.6/31.4	24.5/33.2
45–50	27.1/31.7	25.9/33.1	27.1/33.1	26.1/34.0
50–55	27.7/33.4	26.6/33.7	28.1/34.3	26.8/34.5
55–60	27.6/32.5	27.0/33.4	26.9/32.7	27.3/34.8
60–65	28.2/32.9	28.2/36.1	27.9/32.8	28.4/36.2
65–70	28.0/32.6	28.8/35.2	28.4/34.3	29.0/35.5
70–74	27.4/31.3	27.9/34.8	28.5/33.0	28.6/34.0

p50, 50th percentile of BMI; p90, 90th percentile of BMI. BMI, body mass index; WHO, World Health Organization.

phenotype information, comprising 7937 individuals, were analysed. Subject characteristics are summarised in table 1. The participants were 25–74 years of age; about 50% were older than 50 years and about 50% were men. The range of BMI was 14–57 kg/m^2 (S3) or 16–55 kg/m^2 (S4), respectively.

With respect to the genotype distribution, 3.7% of the participants showed the heterozygous G/A genotype in S3, 3.6% in S4. None of the participants was homozygous for the infrequent A allele in S3. In S4, there was a single subject with the A/A genotype (a 33 year old man with BMI 23.89 kg/m^2), who was included in the analysis, but not treated differently from the heterozygous participants. The observed allele frequency of 1.85% agrees with the according to the Hardy-Weinberg equilibrium expected number of 2.7 A/A subjects among the 7937 participants. Mean (SD) BMI was 27.1 (4.6) kg/m^2 and 26.6 (3.9) kg/m^2 among the non-carriers (G/G) and the carriers (A/G), respectively.

Table 2 summarises the frequencies of the genotype G/A by sex and various categories of BMI. The frequency of the G/A genotype was lower among those with above average weight overall and in all subgroups (for each survey and each gender separately). In addition, if other groups of BMI (obese versus non-obese, WHO categories) are considered, there was a clear trend towards lower G/A frequencies in the obese or in the higher WHO categories overall and in most of the subgroups (by sex and survey), except for the men in S4.

The two surveys were not only homogeneous in terms of the genotype distribution, but also in the age and sex dependency of BMI—that is, the BMI depended on age and sex in a similar manner in both surveys. In fig 2, we visualised the mean BMI per age group by sex separately for both surveys. This clearly indicates an upward age trend in BMI for both sexes, which is very similar in both surveys. In the younger age groups, women exhibited lower BMIs than men, but showed a steeper increase matching the male BMI at the age of about 50 years. Therefore, we allowed for an extra increase in BMI per age group for women in the regression analyses, which proved to be highly statistically significant ($p<0.0001$). Furthermore, the S4 (conducted 1999/2001) BMI levels were higher, particular in the younger age groups, than the S3 (1994/1995) BMI levels.

The results of the association analysis are summarised in table 3. Firstly, linear regression of the combined data ($n = 7939$) with BMI as continuous outcome showed a significant decrease in BMI of −0.52 BMI points (95% CI −0.02 to −1.03, $p = 0.043$) for the G/A genotype compared with the wildtype (G/G). The trend was observed in almost all investigated subgroups (for each survey and each gender), but was not significant in any of the subgroups.

Table 3 also states the estimates of the covariates sex, study, and age group, and the additional effect of the age group for women in the model, which showed: (*a*) an increase of 2.21 BMI points for men ($p<0.0001$); (*b*) an increase of 0.36 BMI points per 5 years of age ($p<0.0001$); (*c*) an additional increase of the slope of the BMI–age relationship of 0.29 for women, yielding a total increase of about 0.7 BMI points per 5 years of age for women ($p<0.0001$); and (*d*) an increase of 0.28 ($p<0.003$) BMI points for the survey effect of S4 (compared with S3). All estimates of these covariates were highly significant and homogenous across the surveys. The increase of BMI in survey S4 compared with S3 was statistically significant ($p = 0.003$), as suggested by comparison of fig 2A and B.

Even with different modelling of the age, sex, or survey effects, the genotype effect remained unaltered. None of the explored interactions other than the aforementioned modifying effect of sex on the age–BMI relationship were present; there was no heterogeneity of the genotype effect in the two surveys, and no interaction between genotype and age or sex.

Table 2 Genotype frequencies for the surveys S3 (1994–1995) and S4 (1999–2001)

	Genotype frequencies		
	All	**Men**	**Women**
S3			
Number of subjects	4071	2019	2052
% with G/A* (n)			
All	3.7 (152)	4.5 (90)	3.0 (62)
By below/above average weight			
BMI < p50† (n = 2026)	4.2 (86)	5.2 (52)	3.3 (34)
BMI ⩾ p50 (n = 2045)	3.2 (66)	3.7 (38)	2.7 (28)
By obesity status			
BMI <30 (n = 3222)	4.1 (131)	4.8 (78)	3.3 (53)
BMI ⩾30 (n = 849)	2.5 (21)	3.1 (12)	2.0 (9)
By BMI WHO category			
BMI <18.5 (n = 22)	4.5 (1)	0.0 (0)	6.2 (1)
BMI 18.5–25 (n = 1423)	3.6 (51)	5.6 (30)	2.4 (21)
BMI 25–30 (n = 1777)	4.4 (79)	4.4 (48)	4.5 (31)
BMI 30–35 (n = 648)	2.8 (18)	3.0 (10)	2.5 (8)
BMI 35–40 (n = 156)	1.9 (3)	3.7 (2)	1.0 (1)
BMI 40+ (n = 45)	0 (0)	0 (0)	0 (0)
S4			
Number of subjects	3866	1919	1947
% with G/A or A/A‡ (n)			
All	3.6 (139)	3.7 (72)	3.4 (67)
By below/above average weight			
BMI < p50† (n = 1926)	4.1 (79)	4.0 (38)	4.2 (41)
BMI ⩾ p50 (n = 1940)	3.1 (60)	3.5 (34)	2.7 (26)
By obesity status:			
BMI <30 (n = 2955)	3.8 (112)	3.7 (55)	3.9 (57)
BMI ⩾30 (n = 899)	3.0 (27)	4.0 (17)	2.1 (10)
By BMI WHO category			
BMI <18.5 (n = 24)	0.0 (0)	0.0 (0)	0.0 (0)
BMI 20–25 (n = 1273)	4.1 (53)	3.7 (18)	4.5 (35)
BMI 25–30 (n = 1670)	3.5 (59)	3.7 (37)	3.2 (22)
BMI 30–35 (n = 668)	3.3 (22)	3.8 (13)	2.8 (9)
BMI 35–40 (n = 172)	2.3 (4)	5.0 (3)	0.9 (1)
BMI 40+ (n = 59)	1.7 (1)	4.3 (1)	0 (0)

*No subject with A/A; †p50 = age, sex, and survey specific 50th percentile of BMI; ‡one subject with A/A.

Secondly, using a logistic regression model, we computed OR estimates comparing above average weight participants (individuals with BMI ⩾ the age, sex, and study specific median BMI, n = 3985) versus below average weight participants (n = 3952). For this analysis, the covariate effects are not shown as they were all practically zero owing to the fact that age, sex, and survey specific cutoff points were chosen. A significantly lower OR of 0.75 (table 3, 95% CI 0.59 to 0.95, $p = 0.017$) was estimated. As the definition of above average weight was made by the age, sex, and survey specific median BMI, no age, sex, or survey effects remained in this comparison. The association was apparent not only for above average weight participants, but also for obese subjects; comparing individuals with BMI ⩾30 versus BMI <30 yielded a significantly decreased OR of 0.69 (95% CI 0.50 to 0.96, $p = 0.026$).

DISCUSSION

MC4R as a candidate gene

Genetic studies have pointed to the importance of the melanocortin system in several complex human pathways such as pigmentation, severe hyperinsulinaemia, lipolysis, food intake, thermogenesis, sexual behaviour, memory, and inflammatory response.[3 5 7–10] Because MC4R is part of the melanocortinergic pathway that controls energy homeostasis and plays a key role in body weight regulation, the corresponding gene is a likely candidate for obesity.

The major gene effect of the *MC4R* in obesity is well known;[1 8 13 19] up to 6% of obese individuals show a functionally relevant mutation in this gene.[2 3] These mutations often result in BMIs larger than 30 kg/m^2 due to either

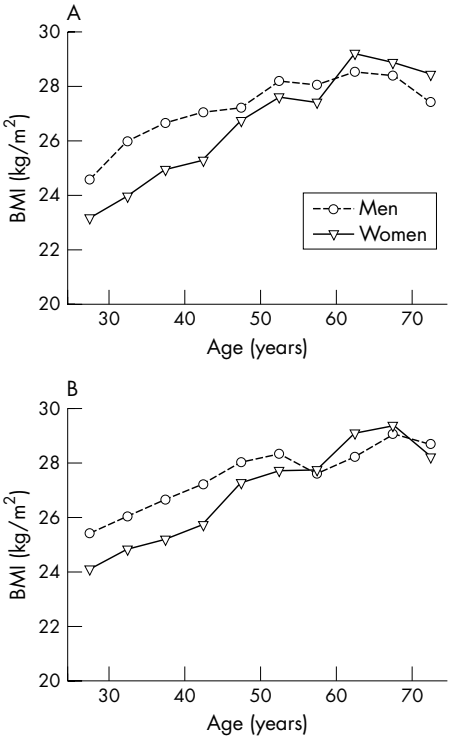

Figure 2 Age–BMI relationship by gender and survey: mean BMI for each 5 year age group for men (solid line) and for women (broken line) (A) survey S3 (1994/1995), (B) survey S4 (1991/2001). It is clear that this relationship is homogeneous for both surveys. An intercept can be observed for men versus women and a secular trend with higher BMIs in the later survey (S4) can be seen particularly for the 25–60 year olds.

loss of function or reduced function. Recently, the melanocortins and their receptors have become the target for drug based treatment of human physiological processes; for example, *MC3R* and *MC4R* are likely targets for controlling body weight. Because functional differences between the isoleucine comprising and the wildtype receptor have not been detected,[1 12 20] it is unclear if the isoleucine variant in itself underlies our finding. Therefore, we cannot exclude that the V103I variant is in linkage disequilibrium with an unidentified causative functional polymorphism in the 5′ or 3′ region of the *MC4R*.[4 11]

It was the aim of this investigation to clarify the association between the *MC4R* 103I variant and decreased body mass in a general white population and to provide clear evidence on the extent of this decrease. The presented pooled analysis of two population based surveys comprising 7937 participants confirmed and extended previous findings of a negative association of the G/A genotype of the *MC4R* V103I polymorphism and obesity.

Association of the 103I with decreased BMI

We found a significant decrease in BMI of 0.52 kg/m^2 by the G/A *MC4R* genotype (95% CI −1.03 to −0.02, p = 0.043). Our study is the first to provide a significant estimate of the association of the 103I variant with a quantitative measure of obesity and is the first singular study of considerable size to prove such an association. To our knowledge, it is one of the largest population based association studies on genetic effects on obesity to date investigating a homogeneous population. A similar estimate of −0.48 kg/m^2 (p = 0.22) was described previously, but lacked power and consequently statistical significance.[4] Analysing the subgroups (each survey and each gender separately) using sensitivity analyses, the same tendency was found, but given the small effect size, the results were not significant due to the smaller subgroup sample sizes. For both surveys combined, the G/A genotype effect estimate for women was double that for men (−0.74 kg/m^2 versus −0.35 kg/m^2) being in line with a gender effect for *MC4R* mutations as described by Dempfle *et al*.[19] However, the gender difference was not significant overall and in fact only pronounced in S4, but not in S3 (table 3).

Negative association of 103I with above average weight and obesity

We found a significant association of the G/A *MC4R* genotype with being above average weight (defined as exceeding the age, sex, and survey specific median BMI compared with being below average weight) (OR 0.75, 95% CI 0.59 to 0.95, p = 0.017) as well as with being obese (defined as BMI ⩾30 kg/m^2 according to the WHO definition) compared with being non-obese (defined as BMI <30 kg/m^2) (OR 0.69, 95% CI 0.50 to 0.96, p = 0.026). In two respects, our results extend the finding from the meta-analysis,[4] which revealed an OR of 0.69 (95% CI 0.50 to 0.96, p = 0.03) comparing the obese and extremely obese with normal subjects in a total of 7713 participants from 14 studies in two ways. Firstly, our results confirm that the meta-analysis effect did not result from publication or selection bias by finding a comparable effect in a population based setting with 7937 subjects from one study. Secondly, being able to show significantly reduced ORs comparing subjects with above average BMI with those lower than the average indicates that the effect does not apply to only the extreme groups. The G/A genotype was observed in 3.7% of the participants in both of our surveys, which is in line with previous reports on white populations.[12–15]

Age, sex, and survey effects

Regarding the age and sex effects on BMI, we observed (*a*) a very homogeneous increase of BMI by age of 0.3 kg/m^2 per 5 years of age for men and approximately 0.7 kg/m^2 for women across the two surveys, and (*b*) a higher BMI for men (table 3, fig 2). Further, we found a highly significant survey effect of +0.3 kg/m^2 for S4 compared with S3, indicating that the 25–75 year old population of the study region of the years 1999–2001 had on average an increased BMI of +0.3 kg/m^2 compared with the age matched population of the same region 5 years ealier (1994/1995), a result in accordance with the trend towards increasing BMI observed in many countries. These data may illustrate the impact of the change in lifestyle on BMI, as the two surveys are sampled from the same population at two different points in time and a change in the genetics of this population is highly unlikely.

Limitations and strengths of the study

Regarding limitations of our study, it needs to be noted that our study shares 2334 (383 obese cases and 1951 controls; 29% of the data presented here) participants with the stated meta-analysis.[4] Altogether, that meta-analysis was based on 7713 subjects (3631 cases and 4082 controls). However, neither the estimates of the meta-analysis nor those of our study presented here depend only on the common 2334 subjects: similar results (albeit non-significant) were also found analysing survey S3 alone (n = 4071), which was not part of the meta-analysis. Among the 14 studies in the meta-analysis, 11 studies showed a negative association (including the 2334 subjects of S4). The OR estimates of the 11 studies for which the OR was computable, were 0.30, 0.55, 0.58, 0.58, 0.67, 0.69, 0.74, 0.80, 0.92, 1.08, and 1.28. Therefore, the OR estimate of the 2334 subjects from our study of 0.58 was not an extreme one.

The present study has the advantage of being able to use a homogeneous definition of above average weight and obesity,

Table 3 Association of the MC4R V103I G/A genotype (compared with the wildtype G/G) with BMI as continuous variable via linear regression and with being above average weight (BMI ⩾ age, sex, and survey specific median BMI) via logistic regression

		Parameter estimate		
Study	**Parameter**	**All†**	**Men‡**	**Women‡**
Unit change in BMI (slope) in kg/m^2				
S3+S4 (n=7937)	G/A* genotype *v* G/G	**−0.52 (−1.03 to −0.02) (p=0.043)**	−0.36 (−0.95 to 0.23) (p=0.23)	−0.74 (−1.59 to 0.11) (p=0.09)
	Age group all	**0.36 (0.31 to 0.41) (p<0.0001)**	0.36 (0.32 to 0.40) (p<0.0001)	0.65 (0.59 to 0.70) (p<0.0001)
	Male	**2.21 (1.79 to 2.63) (p<0.0001)**	–	–
	Age group for women	**0.29 (0.22 to 0.36) (p<0.0001)**	–	–
	S4 study effect	**0.28 (0.09 to 0.48) (p=0.003)**	0.20 (−0.03 to 0.43) (p=0.10)	0.37 (0.07 to 0.67) (p=0.02)
S3 (n=4071)	G/A genotype *v* G/G	−0.61 (−1.29 to 0.06) (p=0.08)	−0.73 (−1.49 to 0.02) (p=0.06)	−0.45 (−1.64 to 0.75) (p=0.46)
	Age group all	0.36 (0.29 to 0.42) (p<0.0001)	0.36 (0.30 to 0.41) (p<0.0001)	0.68 (0.60 to 0.75) (p<0.0001)
	Male	2.47 (1.91 to 3.04) (p<0.0001)	–	–
	Age group for women	0.32 (0.23 to 0.42) (p<0.0001)	–	–
S4 (n=3866)	G/A* genotype *v* G/G	−0.44 (−1.20 to 0.32) (p=0.25)	−0.11 (−0.82 to 1.03) (p=0.82)	−1.03 (−0.82 to 1.03) (p=0.10)
	Age group all	0.36 (0.29 to 0.44) (p<0.0001)	0.36 (0.30 to 0.43) (p<0.0001)	0.62 (0.54 to 0.70) (p<0.0001)
	Male	1.94 (1.31 to 2.56) (p<0.0001)	–	–
	Age group for women	0.26 (0.15 to 0.36) (p<0.0001)	–	–
Relative change of odds of being above average weight (OR)				
S3+ S4	G/A* genotype *v* G/G	**0.75 (0.59 to 0.95) (p=0.017)**	0.78 (0.57 to 1.08) (p=0.133)	0.71 (0.49 to 1.01) (p=0.056)
S3	G/A genotype *v* G/G	0.75 (0.54 to 1.04) (p=0.093)	0.71 (0.46 to 1.09) (p=0.122)	0.81 (0.49 to 1.35) p= (0.418)
S4	G/A* genotype *v* G/G	0.75 (0.53 to 1.05) (p=0.088)	0.88 (0.55 to 1.41) (p=0.608)	0.62 (0.38 to 1.02) (p=0.062)

Results are the beta estimate (linear regression), or the odds ratio estimate (logistic regression), with 95% confidence interval and p values in parentheses. *The one subject from S4 with A/A genotype is collapsed into the G/A genotype group; †full model: adjusted for study, sex, age groups assuming a trend per group and an additional change in the BMI–age group relationship for women; ‡sex specific model, adjusted for study, age groups assuming a trend per group. The main results (S3+S4 together) are **bold**.

whereas it was a limitation of the meta-analysis noted by the authors themselves that they used quite heterogeneous definitions across the 14 studies (using cutoff points of 28, 30, 35, or 40 kg/m^2, or 90th BMI percentile for the cases and cutoff points of 25, 22, or 30 kg/m^2 or the 50th BMI percentile for the controls). Further, our study overcomes the potential danger of publication bias inherent in any meta-analysis. Our study is large and population based, pooling the data of two highly homogeneous surveys and addressing age and sex effects on BMI as well as a secular trend between the years 1994–1995 and 1999–2001. Finally, our study uses the full range of BMI in both the quantitative and qualitative analyses, whereas subjects with intermediate BMI were excluded from the meta-analysis.

Summary

In summary, the present study confirms and extends previous findings of a negative association of the *MC4R* 103I variant and obesity. We were able to confirm this association in a population based sample comprising 7937 individuals and for the first time to provide significant evidence of a decrease of 0.52 BMI points in subjects with the G/A genotype. Our results substantiate that genetic variation in the *MC4R* can be associated with both an elevated (mutations) and reduced (V103I polymorphism) body weight. The V103I polymorphism can be viewed as contributing to polygenetically regulated body weight.

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