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Association of the melanocortin-4 receptor V103I polymorphism with dietary intake in severely obese individuals

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Abstract

Background—Several studies have reported that carriers of the 103I allele of the *MC4R* gene had lower body weight when compared to the wild-type genotype. A recent study found an association of the *MC4R* 103I variant with carbohydrate intake which possibly mediates some of the association of this variant with leanness.

Objective—The purpose of our study was to investigate the association between the *MC4R* V103I polymorphism and dietary intake derived by the Willett food frequency questionnaire in individuals with severe obesity.

Design—The *MC4R* V103I polymorphism was genotyped in a group of 1029 severely obese white subjects with an average BMI of 46.0 kg/m² (range 33–92 kg/m²).

Results—Carriers of the 103I allele showed a significantly higher daily energy (+364 kcal/day or +19%, p=0.03) and carbohydrate intake (+57 g/day or +27%, p=0.01), but there was no relationship with BMI. No notable association of this polymorphism with lipid and glucose parameters of the metabolic syndrome was observed as indicated in a previous study.

Conclusions—The higher dietary intake of carbohydrates in severely obese individuals with the *MC4R* 103I variant is in line with previous findings and might indicate a differential consequence on body size measures in extremely obese subjects when compared to the general population.

Keywords

melanocortin-4 receptor (MC4R); genetic association; obesity; lipoprotein metabolism; dietary intake

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Introduction

The melanocortin-4 receptor (*MC4R*) gene is highly expressed in the hypothalamus where the central control of feeding and energy balance is located (1). Whereas loss-of-function mutations of this gene cause monogenic forms of obesity, several mutations which do not disrupt the gene have an uncertain influence on weight regulation and obesity. One of the most common and widely investigated *MC4R* polymorphisms is the V103I missense variant which has a relatively low population frequency of 2–4%. Several studies including meta-analyses have investigated the influence of this variant on BMI and revealed that heterozygote carriers of V103I might have an 18–30% lower risk for obesity (2–6). Interestingly, a recent study found an association of the *MC4R* 103I variant with carbohydrate intake of borderline significance which possibly mediates some of the association of this variant with leanness (7).

In the present study we investigated whether the *MC4R* 103I variant shows an association with weight by influencing the amount and quality of food intake in severely obese subjects using high-quality information on energy and carbohydrate intake.

Subjects and Methods

The study included 1029 white individuals of the same geographical region of Utah recruited for severe obesity with BMI between 33 and 92 kg/m² who have been described in detail elsewhere as part of recent studies (8;9). Briefly, the investigated individuals were either seeking gastric bypass surgery or were randomly chosen from a population-based sample of severely obese participants not seeking gastric bypass surgery. The examination of patients undergoing gastric bypass surgery was done prior to the intervention.

Twenty-one percent of the patients belonged to obesity class II (35 – <40 kg/m²) and 78% to the obesity class III ((≥40 kg/m²). The daily amount of energy intake (kcal/day) including the total amount of fat, carbohydrates and proteins were derived from the Willett food frequency questionnaire (10) in 926 severe obese subjects. Informed consent was obtained from all subjects. Bioelectrical impedance equipment (RJL Systems Analyzer, Quantum II, Clinton, MI) was used to determine the percentage body fat.

Laboratory Methods

Blood samples were collected after an overnight fasting period. Lipid and lipoprotein concentrations were measured as described in detail (11). Genotyping of the *MC4R* V103I polymorphism (rs2229616) was done within the Genotyping Unit of the Gene Discovery Core Facility at the Innsbruck Medical University, Austria, using a 5′ nuclease allelic discrimination (Taqman) assay in all subjects with sufficient amount and quality of DNA. All carriers of the 103I allele and a similar number of the wildtype genotypes were verified by sequencing. Genotypes were available in 1029 individuals of the Utah study group with a genotyping success rate of 99.2%.

Statistical Analysis

An age- and gender-adjusted general linear regression model was used to estimate the association of the genotypes of the *MC4R* V103I polymorphism with quantitative phenotypes as dependent variables. We excluded those subjects with values above or below 4 standard deviations (SD) of the respective continuous variables to control for outliers. We had 80% power at a 5% type 1 error rate to detect a difference in total daily amount of energy consumed of about 500 kcal and a difference in daily carbohydrate intake of 69 g/day between carriers and non-carriers of the variant (corresponds to about 25% and 32%, respectively, of the daily intake).

Results

Clinical characteristics and laboratory data of the study participants are reported in Table 1.

The primary analysis investigated the daily age- and sex-adjusted dietary intake and observed that carriers of the 103I variant had on average a 19% higher total energy intake (+364 kcal/day, p=0.03) compared to homozygote wildtypes (Table 2). A major part of this higher energy intake was due to a 27% higher carbohydrate intake (+57 g/day, p=0.01) and a slightly 15% higher fat intake (+10.7 g/day, p=0.13) (Table 2). Since the association between the *MC4R* V103I polymorphism and total energy intake adjusted for carbohydrate intake was no longer significant (p=0.83), this indicates that the entire association of total energy intake is explained by the carbohydrate intake. The association between the 103I variant and carbohydrate intake remained significant (p=0.01) and estimates persisted, when we adjusted additionally for BMI.

Because 81.4% of the individuals were women, we performed a sensitivity analysis by excluding all men from the analysis. We observed even stronger associations for total calories (+409 kcal/day, p=0.01) and especially carbohydrate intake (+64 g/day, p=0.005) for 103I variant carriers.

In the secondary analysis we did not detect a significant association between the *MC4R* V103I polymorphism and BMI with estimates 1.89 kg/m² higher for the heterozygote genotype carriers compared to the wildtype carriers (p=0.17) (Table 2). When we adjusted this analysis for carbohydrate intake or daily energy intake, the BMI estimates became even weaker (+1.10 kg/m², p=0.44). When we extended the analysis to other measures of body size and lipid and glucose metabolism we observed that waist and percentage body fat in carriers of the 103I variant pointed in the same direction as BMI, however, without being significant. We did not observe any significant association of this polymorphism with lipid and glucose metabolism.

Discussion

A recent population-based study described a borderline significantly higher carbohydrate intake for heterozygote *MC4R* V103I genotype carriers (7). Since a carbohydrate-rich diet is associated with a leaner body composition (12), it was speculated that this polymorphism plays a role in appetite regulation in humans by reducing the quantity or modifying the quality of food intake. This might modulate central obesity, as well as lipid and glucose metabolism. In line with these findings (7) we observed a significantly higher carbohydrate intake in rare allele carriers in our study with severely obese subjects. However, these genotype carriers had also a general increase in energy intake which might explain why we did not find a lower BMI as reported in the literature. Previous studies in population-based subjects or spanning the entire range of BMI showed a lower BMI in rare allele carriers (2–6). This association might be disturbed in severe obese subjects as seen in our study as well as in the only other large studies investigating severely obese persons of obesity class II and III (5;13).

It might be speculated that the rare allele of the *MC4R* V103I may modulate appetite towards higher carbohydrate intake, which may have strongly differential effects on different population groups. For the general population, there may be a tendency towards a leaner phenotype being associated with increased carbohydrate intake, while for a subgroup in already a severe obesity stage, this appetite modulation is connected with higher total energy intake counteracting a leaner phenotype. According to mediator analysis proposed by Prentice et al. (14), the to some part mediating role of carbohydrate intake is supported by the analysis of BMI adjusted for carbohydrate intake. This analysis yielded decreased

association estimates for BMI which supports the idea that the influence of this polymorphism on body size measures is mediated by the nutritional behavior.

Even though our study included 1029 mostly severely obese patients, one might assume that our findings might be limited due to the small number of 103I genotype carriers as the variant is rather rare. While we had 70% power for the observed higher intake of carbohydrates, our power to detect a BMI difference of 1.5 kg/m² was only 20%. Furthermore, the nutritional variables are very difficult to assess and usually involve substantial uncertainty (15), which aggravates the power issue. However, statistical theory teaches that such random uncertainty in the regression outcome variable would not impose a bias on the estimate, as the uncertainty can be considered to be undifferential between variant carriers and non-carriers. Therefore, the association estimate with the true underlying carbohydrate intake could be expected to be the same as observed but with smaller confidence intervals and therefore better p values.

Underreporting of dietary intake in obese individuals is also a well-known problem. However, as we analyze a “case-only group”, systematic underreporting could be accounted for by subtracting a constant from the outcome variable values and would neither result in biased estimates nor in a loss of precision, as the underreporting can be considered to be independent of the genotype status (15).

Finally, it is highly unlikely that our finding is a technical artifact of genotyping since we confirmed each 103I genotype carrier and a similar number of wildtype genotype carriers by sequencing.

We propose that future large-scale studies may especially analyze the association of *MC4R* polymorphisms stratified by severity of obesity. If our preliminary finding can be confirmed, we propose a differential influence of this polymorphism in severely obese persons compared to the general population. It is conceivable that several interconnected mediators in appetite regulation are heavily disturbed in severely obese individuals which changes the influence of this polymorphism on the ingested nutritional spectrum.

In summary, the higher dietary intake of carbohydrates in severely obese individuals with the *MC4R* 103I variant is in line with previous findings and might indicate a differential consequence on body size measures in extremely obese subjects when compared to the general population which, however, needs confirmation in independent studies or meta-analyses.

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Table 1

Clinical and laboratory data of 1029 severe obese individuals from Utah.

Age, yrs	44.3±11.4
Gender: male/female, n (%)	191/838 (18.6/81.4)
Total energy intake, kcal/day [a]	2076±938
Total carbohydrates, g/day [a]	240±125
Total fat, g/day [a]	84±42
Total protein, g/day [a]	95±40
Body mass index, kg/m²	46.0±7.6
Waist, cm	134±18
Waist-hip ratio	0.96±0.09
Body fat, %	49.1±6.4
Total cholesterol, mg/dL	187±36
LDL cholesterol, mg/dL	108±27
HDL cholesterol, mg/dL	46±11
Triglycerides, mg/dL	186±106
Hypertension, n (%)	619 (60.2)
Systolic blood pressure, mmHg	127±18
Diastolic blood pressure, mmHg	72±11
Glucose, mg/dL	104±33
Diabetes mellitus, n (%)	223 (21.7)

Values derived from descriptive data analysis are provided as mean and standard deviation if not indicated otherwise.

[a] Measures of dietary intake are available in 926 of the 1029 individuals.

Table 2

The association of the MC4R V103I polymorphism with age- and sex-adjusted measures of dietary intake (primary analysis) as well as measures of body composition, lipoprotein and glucose metabolism (secondary analysis) in a group of 1029 severely obese individuals.

	Genotypes			
	GG (n=1001)	GA (n=28)	P value	Difference in means GA versus GG
Primary analysis				
Total energy intake, kcal/day [a, b]	1896±1.01	2260±1.08	0.03	+364
Total carbohydrates, g/day [a, b]	213±1.02	270±1.10	0.01	+57
Total fat, g/day [a, b]	75±1.02	86±1.09	0.13	+10.7
Total protein, g/day [a, b]	88±1.01	94±1.09	0.41	+6.3
Secondary analysis				
BMI (kg/m²) [a]	45.31±1.00	47.20±1.03	0.17	+1.89
Waist, cm	134±0.54	137±3.23	0.39	+2.81
Body fat, % [a]	48.7±1.00	49.2±1.02	0.55	+0.50
Total cholesterol, mg/dL	186±1.12	183±6.67	0.60	−3.53
LDL cholesterol, mg/dL	108±0.87	104±5.16	0.40	−4.39
HDL cholesterol, mg/dL	46±0.32	47±1.93	0.65	+0.90
Triglycerides, mg/dL [a]	165±1.02	158±1.09	0.65	−6.66
Glucose, mg/dL [a]	97±1.01	98±1.03	0.96	+0.18

Values derived from general linear regression models are provided as age and sex-adjusted mean±standard errors; Individuals with values ±4SD of the mean of the particular variables of the group are excluded from the analysis.

[a] These values were computed on the log-scale and retransformed for tabulation.

[b] Measures of dietary intake are available in 926 individuals (900 GG and 26 GA genotypes).