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Common Variants Near *MC4R*: Exploring Gender Effects in Overweight and Obese Children and Adolescents Participating in a Lifestyle Intervention*

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Keywords
MC4R · Obesity · BMI-SDS reduction · Metabolism markers · Insulin

Summary
Objective: Association with obesity and increased insulin levels have been reported for two variants (rs17782313 and rs12970134) located downstream of the melanocortin-4 receptor gene (*MC4R*). **Methods:** We investigated whether these variants have sex-specific effects on overweight, obesity and 14 related phenotypes in 889 overweight and obese children and adolescents. We also explored the impact of the variants on weight change in 367 of the 889 cases who participated in an intervention program. Prior to these analyses we showed that both variants were associated with overweight/obesity in the analyzed 889 cases versus 442 normal-weight and lean controls (case-control study). **Results:** In explorative analyses we observed higher diastolic blood pressure levels in males (rs17782313: β = 2.52 mm Hg per risk allele; p = 0.003) but reduced blood pressure level in females for the same risk allele (β = –1.72 mm Hg; p = 0.039). We also detected a greater BMI standard deviation score (BMI-SDS) reduction in females with the risk allele at rs17782313 (β = 0.086 per risk allele; p = 0.021). Additionally, we observed evidence for an association of the same risk allele with insulin levels (β = 0.029 log (μU/ml); p = 0.044) with no sex-specific effect. For the remaining 11 phenotypes, we observed no evidence for a (sex-specific) association. **Conclusions:** In sum, our data support the associations of variants rs17782313 and rs12970134 near *MC4R* with early onset obesity and increased insulin levels. Exploratory evidence for sex-specific effects of the risk alleles were observed for diastolic blood pressure and BMI-SDS reduction.

Introduction

The melanocortin-4 receptor (MC4R) is part of the central melanocortinergic system and is involved in central regulation of energy homeostasis and body weight [1]. Mutations in the *MC4R* leading to a reduced receptor function are found in 2–4% of extremely obese individuals [2]. Carriers of functionally relevant mutations have a significantly higher BMI than their wild-type relatives, and the observed effect is approximately twice as strong in female than in male mutation carriers [3]. In addition to mutations, the common single nucleotide polymorphisms (SNPs) rs12970134 and rs17782313 located downstream of the *MC4R* (154 kb and 188 kb, respectively) have consistently been shown to be associated with obesity and related traits [4, 5]. Additionally, sex-specific effects of rs17782313 on increased BMI have been suggested [6] although not supported in the original

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large-scale GWAS meta-analysis ($p_{sex.-interaction}$ = 0.11) [4]. Renström et al. [6] described a nominal association of the rs17782313 obesity risk allele with higher BMI in females (β = 0.41 kg/m^2 per copy of the risk allele; p = 0.003) but not in males (β = –0.03 kg/m^2; p = 0.83). However, their observation lacks a confirmation.

Sex-specific effects have been suggested for other GWAS-derived markers. As examples, Jacobsson et al. [7] reported sex-specific effects for the obesity risk alleles in intron 1 of the fat mass and obesity-associated gene (*FTO*). They reported a significant association of the A-allele of SNP rs9939609 with BMI and obesity in females only. More recently, Holzapfel and colleagues [8] described a similar effect for the SNP rs7498665 in *SH2B1* (SH2B adaptor protein 1). Taken together, these studies suggest that variants discovered in recent GWAS for body weight regulation may have sex-specific effects on obesity or obesity-related phenotypes.

As claims of sex-specific effects are frequently spurious [9], our study focused on the validation of sex-specific effects of rs17782313 and rs12970134 positioned near *MC4R*. As a prerequisite for these analyses we first analyzed 889 overweight and obese children and adolescent cases (according to criteria of the International Obesity Task Force (IOTF) [10]) to confirm the obesity effect of the risk alleles in comparison to 442 healthy lean control individuals (case-control design). Secondly, we analyzed sex-specific effects for obesity-related quantitative traits such as insulin resistance and HDL/LDL-cholesterol as assessed among the 889 overweight and obese children and adolescents. Thirdly, we explored the (sex-specific) impact of the two SNPs on weight change after a 1-year lifestyle intervention in a subgroup of the 889 cases.

Participants and Methods

Subjects

Written informed consent was given by all participants and in the case of minors, by their parents. The studies were approved by the Ethics Committees of the Universities of Bonn, Witten/Herdecke, Essen and Marburg and carried out according to the Declaration of Helsinki.

The study group comprised 889 overweight and obese children and adolescents (mean age ± SD 10.69 ± 2.98 years; 473 females (53.2%); mean BMI 28.09 ± 5.19 kg/m^2; mean BMI standard deviation score (BMI-SDS) according to *www.mybmi.de* [11] 2.46 ± 0.54). A total of 765 subjects (86.1%) had a BMI above the age- and sex-specific 97th percentile (IOTF [10]). The overweight and obese children and adolescents were recruited by the Department of Pediatrics, University of Bonn, Germany [12], and Vestische Hospital for Children and Adolescents, University of Witten/Herdecke, Datteln, Germany [13].

The control group comprised 442 healthy lean individuals who were ascertained at the University of Marburg, Germany, as described previously [14] (mean age 18.31 ± 1.10 years; 271 females (61.3%); mean BMI 18.31 ± 1.10 kg/m^2; mean BMI-SDS –1.38 ± 0.35). Note that our control group is older than the group of cases which should reduce the chances of misclassification errors as opposed to the use of lean children and adolescents because younger controls might become overweight or obese later on in life. Moreover, our controls reported to have never been overweight or obese earlier in their lives as assessed by interview [14].

Follow-Up Study

A subset of the 889 obese cases – 367 overweight and obese individuals (mean age 10.77 ± 2.66 years; 205 females (55.9%); mean BMI 27.65 ± 4.66 kg/m^2; mean BMI-SDS 2.40 ± 0.50) – took part in the outpatient lifestyle intervention 'Obeldicks' program at the Vestische Hospital for Children and Adolescents, University of Witten/Herdecke, Datteln, Germany [15]. Briefly, this 1-year intervention program for obese children is based on physical exercise, nutrition education, and behavior therapy including individual psychological care of the child and his or her family. BMI was measured at baseline and at the end of intervention.

Blood Parameters

In up to 889 overweight and obese children and adolescents fasting blood parameters for several lipid metabolism markers, such as triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol as well as glucose, and insulin levels were obtained. Blood samples were taken in the morning after an overnight fast. Plasma levels of triglycerides, total cholesterol, HDL- and LDL-cholesterol, insulin, and glucose were measured using commercially available test kits (Roche Diagnostics, Mannheim, Germany; Boehringer, Mannheim, Germany; Ortho Clinical Diagnostics, Neckargemünd, Germany; Abbott, Wiesbaden, Germany). Intra- and inter-assay variations of these variables were less than 5%. Homeostasis model assessment (HOMA) was calculated as follows: resistance (HOMA) = insulin (mU/l) × glucose (mmol/l) / 22.5 [16].

Anthropometric Parameters

Body height was measured in cases and controls to the nearest centimeter using a rigid stadiometer. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. The degree of overweight was quantified using Cole's least mean square method, which normalizes the BMI-skewed distribution in childhood and expressed BMI as a standard deviation score (BMI-SDS) [17]. German population-based reference data were used for body height, weight and BMI [9]. Overweight was defined according to the guidelines of the IOTF [10] by using the national BMI percentiles assuming 15% overweight (≥85th percentile) including 5% obesity (≥95th percentile) in 1990. Blood pressure was measured according to the guidelines of the National High Blood Pressure Education Program (NHBPEP) [18].

Genotyping

We used TaqMan® SNP genotyping assay (for rs17782313: C_32667060_10 and for rs12970134: C_3058722_10 assays; Applied Biosystems, Darmstadt, Germany) with standard conditions. For validity of genotypes, allele determinations were rated independently by at least two experienced individuals. Discrepancies were either resolved unambiguously or genotyping was repeated; call rates were > 99%. Additionally, 93 individuals were genotyped in duplicate; concordance was 100%. We observed no evidence for deviations from Hardy Weinberg equilibrium (all exact two-sided p values >> 0.05).

Statistics

In the case-control study association analyses were performed using the exact Cochran-Armitage trend test with a linear trend. In the overweight and obese cases the measures of the blood parameters (total cholesterol, HDL- and LDL-cholesterol, triglycerides, insulin and glucose) were $\log_{10}$-transformed to address the skewness of their distributions. Analyses of all quantitative variables (BMI-SDS, BMI-SDS reduction, waist circumference, weight, height, blood pressure, HOMA and the log-transformed blood parameters) were performed using a linear regression with sex and age as covariates.

In the follow-up analysis of BMI-SDS change, BMI-SDS at the beginning of the intervention was included as additional covariate. All analyses were performed using a (log-)additive genetic model with the C-allele at rs17782313 and the A-allele at rs12970134 coded as risk alleles following the findings from the literature [4, 5].

To assess possible sex interactions, we also extended the respective models by including a sex × SNP interaction factor in the model. For all variables, confidence intervals were calculated with coverage of 95% (95% CI). Unless otherwise stated, all reported p values are nominal, two-sided and not adjusted for multiple testing.

Power calculations were done with the software QUANTO Version 1.2.4 (*http://hydra.usc.edu/gxe*) for common variants, using an estimated minor allele frequency (MAF) of 0.3 and $\alpha = 0.05$ (two-sided).

For the sample of 889 cases and 442 controls, the power estimates were larger than 80% to detect a log-additive genotype relative risk of 1.28. A log-additive genotype relative risk of 1.38 and 1.47 was detectable with a similar power in the sex-stratified analyses of females and males, respectively. For the quantitative analyses in the sample of 889 (473 females) overweight and obese children and adolescents, the power estimate was larger than 80% to detect an additive effect of 0.08 (0.20 in females, 0.22 in males) in units of SD of a standard normal distribution (standardized effect size). Under the same scenario, the power estimate was larger than 80% to detect a standardized effect size of 0.12 (changes in BMI-SDS; 0.30 in females, 0.34 in males) for the subsample of n = 367 (205 females) overweight and obese children and adolescents who participated in the outpatient lifestyle intervention. Thus, all samples were well powered to detect strong effect sizes of disease-predisposing variants; moderate or smaller effects might have been missed.

Results

We genotyped the SNPs rs17782313 and rs12970134 and performed sex-specific case-control as well as quantitative trait analyses in cases. As both markers were in strong pair-wise linkage disequilibrium ($r^2 = 0.8$), we decided to describe in 'Results' the findings for rs17782313 only, given the larger sample size and focus on BMI in [4] for this SNP (for completeness the results of rs12970134 are given in the tables 1–4; see also supplementary figure 1 at *http://content.karger.com/ProdukteDB/produkte.asp?doi=324557*).

Case-Control Study

The comparison of overweight and obese children and adolescents with healthy lean controls resulted in similar effect size estimates in females ($OR_{TC} = 1.32$, 95% CI 1.03–1.70; $OR_{CC} = 1.74$, 95% CI 1.06–2.90; p = 0.029) and males ($OR_{TC} = 1.50$, 95% CI 1.12–2.03; $OR_{CC} = 2.25$, 95% CI 1.26–4.10; p = 0.006; table 1), which was also supported by a nonsignificant (p = 0.506) sex interaction in the model. The results in the total sample supported the well known obesity association ($OR_{TC} = 1.40$, 95% CI 1.16–1.70; $OR_{CC} = 1.96$, 95% CI 1.35–2.88; p = 0.0003; table 1).

Quantitative Trait Analyses in Cases

Subsequently, we investigated quantitative traits in the overweight and obese children and adolescents. We observed exploratory evidence for a sex-specific effect for diastolic blood pressure (table 2). In particular, the (obesity) risk allele was associated with evidence for reduced diastolic blood pressure levels in females ($\beta = -1.72$; 95% CI –3.35 to –0.10 mm Hg per risk allele; p = 0.039; table 2), whereas in males the risk allele was associated with higher diastolic blood pressure levels ($\beta = 2.52$: 95% CI 0.89–4.16 mm Hg per risk allele; p = 0.003; table 2; $p = 3 \times 10^{-4}$ for the sex interaction). In addition, we also found exploratory evidence for higher fasting insulin levels in risk allele carriers ($\beta = 0.029$; 95% CI 0.001–0.058 $\log_{10}$ (μU/ml) per risk allele; p = 0.044; upon adjustment for BMI p = 0.058; table 3). In this case, however, we observed no evidence for a sex-specific effect (p = 0.8567 for the sex interaction), and indeed the effect sizes were similar in females and males (females: $\beta = 0.032$; 95% CI –0.010 to 0.073 $\log_{10}$ (μU/ml) per risk allele; p = 0.132; males: $\beta = 0.027$; 95% CI –0.012 to 0.065 $\log_{10}$ (μU/ml) per risk allele; p = 0.178; see also supplementary table at *http://content.karger.com/ProdukteDB/produkte.asp?doi=324557*). For the other explored quantitative traits, such as serum levels of triglycerides, total cholesterol, LDL- and HDL-cholesterol, glucose and HOMA, and the anthropometric variables of waist circumference, weight and height, we observed no evidence for an association with rs17782313 genotype – neither in the sex-stratified analyses nor in the analyses of all individuals (all $p \geq 0.05$; table 3; see also supplementary table at *http://content.karger.com/ProdukteDB/produkte.asp?doi=324557*).

Follow-Up Study in a Subgroup of the Cases

Finally, we explored the subgroup of 367 overweight and obese children and adolescents who participated in the lifestyle weight management intervention 'Obeldicks'. Sex-specific analyses were performed for BMI-SDS at baseline (start of intervention) and for BMI-SDS changes after the intervention. At baseline we observed no evidence for genotype-dependent differences in BMI-SDS (table 4); stratification by sex revealed a nominally higher BMI-SDS in males with obesity risk genotype ($\beta = 0.079$; 95% CI –0.016 to 0.174 per risk allele; p = 0.107) but not in females ($\beta = 0.009$; 95% CI –0.098 to 0.117 per risk allele; p = 0.866; p = 0.438 for the SNP × sex interaction); these results were in accordance with the findings obtained for the whole group. Similarly, we observed no general genotype-dependent BMI-SDS change after the intervention ($\beta = 0.035$; 95% CI –0.014 to 0.084 per risk allele; p = 0.163; table 4) but we found exploratory evidence for a greater BMI-SDS reduction in females ($\beta = 0.086$; 95% CI 0.013–0.159 per risk allele; p = 0.021), whereas in males no evidence for such an effect was observable ($\beta = -0.016$; 95% CI –0.079 to 0.046 per risk allele; p = 0.547; table 4 (see also supplementary figure 1 at *http://content.karger.com/ProdukteDB/produkte.asp?doi=324557*); p = 0.034 for the sex interaction).

Discussion

Our primary goal was to replicate sex-specific effects related to rs17782313 and rs12970134 located downstream of *MC4R*. While we confirmed the obesity association in 889 German overweight and obese children and adolescents and 442 nor-

Table 1. Case- control study – overall and stratified by sex for the SNPs rs17782313 and rs12970134

SNP	Group	Genotypes[1] n (%)			Alleles (%)		Odds ratio (OR) (95% CI)[2]	p value[2]
rs17782313		TT	TC	CC	T	C		
	total overweight and obese children and adolescents	436 (49.5)	356 (40.4)	89 (10.1)	69.7	30.3	OR_{TC} 1.40 (1.16–1.70)	3×10^{-4}
	total underweight controls[3]	254 (58.5)	156 (36.0)	24 (5.5)	76.5	23.5	OR_{CC} 1.96 (1.35–2.88)	
	female overweight and obese children and adolescents	236 (50.3)	192 (40.9)	41 (8.8)	70.8	29.2	OR_{TC} 1.32 (1.03–1.70)	0.029
	female underweight controls3	153 (58.2)	95 (36.1)	15 (5.7)	76.2	23.8	ORCC 1.74 (1.06–2.90)	
	male overweight and obese children and adolescents	200 (48.5)	164 (39.8)	48 (11.7)	68.4	31.6	OR_{TC} 1.50 (1.12–2.03)	0.006
	male underweight controls[3]	101 (59.0)	61 (35.7)	9 (5.3)	76.9	23.1	OR_{CC} 2.25 (1.26–4.10)	
rs12970134		GG	GA	AA	G	A		
	total overweight and obese children and adolescents	415 (47.0)	364 (41.2)	104 (11.8)	67.6	32.4	OR_{GA} 1.28 (1.07–1.54)	0.007
	total underweight controls3	231 (53.3)	170 (39.3)	32 (7.4)	73.0	27.0	OR AA 1.64 (1.14–2.36)	
	female overweight and obese children and adolescents	226 (47.9)	195 (41.3)	51 (10.8)	68.5	31.5	OR_{GA} 1.27 (1.00–1.62)	0.052
	female underweight controls3	142 (54.0)	103 (39.2)	18 (6.8)	73.6	26.4	OR AA 1.61 (1.00–2.63)	
	male overweight and obese children and adolescents	189 (46.0)	169 (41.1)	53 (12.9)	66.5	33.5	OR_{GA} 1.28 (0.97–1.69)	0.086
	male underweight controls[3]	89 (52.4)	67 (39.4)	14 (8.2)	72.0	28.0	OR_{AA} 1.63 (0.94–2.87)	

[1]In each genotype group the exact two-sided p-values for deviations from Hardy-Weinberg equilibrium were >> 0.05.
[2]Two-sided p value using the exact Cochran-Armitage trend test (assuming a linear trend).
[3]The controls were a subsample from Hinney et al. [14] which was also genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0.

Table 2. Quantitative trait analyses in 889 overweight and obese children and adolescents (cases) – results of association of the diastolic blood pressure with the variants rs17782313 and rs12970134

SNP	Group	Genotype	n (%)	Diastolic blood pressure, mm Hg (mean ± SD)	Estimate[1]	95% CI	p value[2]
rs17782313	total	CC	68 (9.91)	67.22 ± 11.96	0.350	–0.813 to 1.513	0.556
		CT	290 (42.27)	66.29 ± 10.94			
		TT	328 (47.81)	66.20 ± 10.66			
	females	CC	31 (8.2)	62.81 ± 11.00	–1.722	–3.347 to –0.097	0.039
		CT	166 (43.92)	66.04 ± 10.82			
		TT	181 (47.88)	67.20 ± 10.83			
	males	CC	37 (12.01)	70.92 ± 11.60	2.524	0.893– 4.155	0.003
		CT	124 (40.26)	66.63 ± 11.14			
		TT	147 (47.73)	64.97 ± 10.35			
rs12970134	total	AA	81 (11.81)	66.64 ± 11.53	0.191	–0.938 to 1.321	0.740
		AG	293 (42.71)	66.35 ± 11.01			
		GG	312 (45.48)	66.25 ± 10.66			
	females	AA	41 (10.85)	63.22 ± 10.98	–1.596	–3.146 to –0.046	0.044
		AG	164 (43.39)	66.06 ± 10.77			
		GG	173 (45.77)	67.32 ± 10.86			
	males	AA	40 (12.99)	70.15 ± 11.16	2.181	0.557 to 3.805	0.009
		AG	129 (41.88)	66.71 ± 11.34			
		GG	139 (45.13)	64.93 ± 10.30			

[1]Effect of one copy of the minor (risk) allele in the additive genetic model as determined by linear regression adjusted for age (or age and sex in the analysis of both sexes).
[2]Two-sided p value.

Table 3. Quantitative trait analyses in 889 overweight and obese children and adolescents (cases) – analysis of obesity-related phenotypes and genotypes at rs17782313 and rs12970134

SNP	Variable	Genotype	n (%)	mean ± SD	Estimate[1]	95% CI	p value[2]
rs17782313	BMI-SDS	CC	89 (10.10)	2.48 ± 0.49	0.010	–0.043 to 0.064	0.708
		CT	356 (40.41)	2.47 ± 0.54			
		TT	436 (49.49)	2.46 ± 0.56			
	waist, cm	CC	55 (11.07)	91.09 ± 13.61	0.629	–0.864 to 2.122	0.409
		CT	213 (42.86)	88.45 ± 13.81			
		TT	229 (46.08)	88.69 ± 14.26			
	weight, kg	CC	89 (10.10)	68.41 ± 21.98	0.004	–0.067 to 0.075	0.910
		CT	356 (40.41)	63.25 ± 21.90			
		TT	436 (49.49)	65.39 ± 23.16			
	height, cm	CC	89 (10.10)	152.26 ± 15.80	0.117	–0.657 to 0.890	0.767
		CT	356 (40.41)	148.29 ± 17.50			
		TT	436 (49.49)	149.80 ± 17.03			
	systolic blood pressure, mm Hg	CC	68 (9.91)	117.00 ± 14.01	–0.538	–2.100 to 1.023	0.500
		CT	290 (42.27)	114.29 ± 14.66			
		TT	328 (47.81)	116.23 ± 15.02			
	diastolic blood pressure, mm Hg	CC	68 (9.91)	67.22 ± 11.96	0.350	–0.813 to 1.513	0.556
		CT	290 (42.27)	66.29 ± 10.94			
		TT	328 (47.81)	66.20 ± 10.66			
	total cholesterol[3]	CC	68 (9.87)	2.22 ± 0.08	0.0001	–0.009 to 0.010	0.980
		CT	291 (42.23)	2.23 ± 0.08			
		TT	330 (47.90)	2.22 ± 0.08			
	triglycerides, mg/dl[3]	CC	68 (9.88)	2.02 ± 0.21	0.017	–0.007 to 0.041	0.167
		CT	291 (42.30)	1.96 ± 0.21			
		TT	329 (47.82)	1.96 ± 0.22			
	LDL-cholesterol, mg/dl[3]	CC	84 (10.27)	2.00 ± 0.11	–0.006	–0.020 to 0.008	0.382
		CT	333 (40.71)	1.99 ± 0.14			
		TT	401 (49.02)	2.00 ± 0.13			
	HDL-cholesterol, mg/dl[3]	CC	85 (10.33)	1.67 ± 0.09	–0.002	–0.012 to 0.008	0.648
		CT	335 (40.70)	1.69 ± 0.10			
		TT	403 (48.97)	1.68 ± 0.10			
	glucose, mg/dl[3]	CC	83 (10.15)	1.93 ± 0.04	0.004	–0.001 to 0.008	0.119
		CT	334 (40.83)	1.93 ± 0.05			
		TT	401 (49.02)	1.93 ± 0.04			
	insulin, µU/ml[3]	CC	81 (10.15)	1.20 ± 0.23	0.029	0.001 to 0.058	0.044
		CT	324 (40.60)	1.11 ± 0.31			
		TT	393 (49.25)	1.12 ± 0.31			
	HOMA, µmol/l × mmol / l2[3]	CC	77 (9.96)	3.94 ± 2.34	0.123	–0.199 to 0.444	0.454
		CT	315 (40.75)	3.58 ± 2.95			
		TT	381 (49.29)	3.59 ± 3.47			

Table 3 continued on next page

mal-weight controls [4, 5], we could not confirm the previously described sex effect [6].

Secondly, we investigated 14 obesity-related quantitative traits for their association with the obesity risk variant at rs17782313 within the aforementioned overweight and obese cases. While we observed no evidence for the previously described association [5] of the MC4R variants with insulin resistance (as tested by HOMA), we detected exploratory evidence for an association of the risk variant with increased insulin levels. Interestingly, hyperinsulinemia has been described as part of the MC4R deficiency clinical phenotype [19–21]. In addition, Chambers et al. [5] reported an associa-

Table 3. Continued

SNP	Variable	Genotype	n (%)	mean ± SD	Estimate[1]	95% CI	p value[2]
rs12970134	BMI-SDS	AA	104 (11.78)	2.43 ± 0.49	0.003	–0.050 to 0.055	0.922
		AG	364 (41.22)	2.49 ± 0.55			
		GG	415 (47.00)	2.45 ± 0.55			
	waist, cm	AA	66 (13.28)	90.48 ±14.61	0.569	–0.869 to 2.006	0.439
		AG	205 (41.25)	88.70 ± 13.78			
		GG	226 (45.47)	88.52 ± 14.02			
	weight, kg	AA	104 (11.78)	67.85 ± 21.61	0.004	–0.065 to 0.073	0.911
		AG	364 (41.22)	63.31 ± 21.91			
		GG	415 (47.00)	65.42 ± 23.33			
	height, cm	AA	104 (11.78)	152.28 ± 15.35	0.305	–0.449 to 1.059	0.429
		AG	364 (41.22)	148.22 ± 17.54			
		GG	415 (47.00)	149.72 ± 17.07			
	systolic blood pressure, mm Hg	AA	81 (11.81)	116.53 ± 14.71	–0.337	–1.854 to 1.179	0.663
		AG	293 (42.71)	114.54 ± 14.66			
		GG	312 (45.48)	116.10 ± 14.92			
	diastolic blood pressure, mm Hg	AA	81 (11.81)	66.64 ± 11.53	0.191	–0.938 to 1.321	0.740
		AG	293 (42.71)	66.35 ± 11.01			
		GG	312 (45.48)	66.25 ± 10.66			
	total cholesterol[3]	AA	82 (11.90)	2.22 ± 0.07	0.001	–0.008 to 0.010	0.880
		AG	293 (42.53)	2.23 ± 0.09			
		GG	314 (45.57)	2.22 ± 0.08			
	triglycerides, mg/dl[3]	AA	82 (11.92)	1.99 ± 0.20	0.016	–0.008 to 0.039	0.188
		AG	293 (42.59)	1.97 ± 0.22			
		GG	313 (45.49)	1.96 ± 0.21			
	LDL-cholesterol, mg/dl[3]	AA	97 (11.82)	2.00 ± 0.11	–0.001	–0.014 to 0.013	0.938
		AG	341 (41.53)	2.00 ± 0.14			
		GG	383 (46.65)	2.00 ± 0.14			
	HDL-cholesterol, mg/dl[3]	AA	98 (11.86)	1.68 ± 0.09	–0.003	–0.013 to 0.007	0.521
		AG	343 (41.53)	1.69 ± 0.10			
		GG	385 (46.61)	1.69 ± 0.10			
	glucose, mg/dl[3]	AA	98 (11.97)	1.93 ± 0.04	0.004	–0.001 to 0.008	0.083
		AG	339 (41.39)	1.93 ± 0.05			
		GG	382 (46.64)	1.93 ± 0.04			
	insulin, µU/ml[3]	AA	96 (11.99)	1.18 ± 0.26	0.033	0.005 to 0.060	0.020
		AG	331 (41.32)	1.12 ± 0.31			
		GG	374 (46.69)	1.11 ± 0.31			
	HOMA, µmol/l × mmol / l2[3]	AA	92 (11.86)	3.86 ± 2.44	0.207	–0.104 to 0.519	0.192
		AG	322 (41.49)	3.67 ± 3.17			
		GG	362 (46.65)	3.50 ± 3.31			

[1]Effect of one copy of the minor (risk) allele in the additive genetic model as determined by linear regression.
[2]Two-sided p value.
[3]Results for the linear regression analyses (for an additive genetic model) of log 10-transformed variables with age (linear) and sex as covariates.

tion of insulin resistance with the risk allele (A) of rs12970134 in a mixed adult sample of Indian Asian (ca. 62%) and European ancestry (ca. 38%). However, three other independent large studies in adults did not support the initial findings for rs17782313 [22, 23] and rs12970134 [23, 24] with fasting insulin levels or insulin resistance. Possible explanations for this inconclusive pattern are differences in the assessed phenotype as well as the possibility that the impact of the variants on promoting insulin resistance is different in children and adolescents compared to adults.

In the sex-stratified analyses we observed exploratory evidence for association of the MC4R risk variants with in-

Table 4. Follow-up study in a subgroup of 367 overweight and obese children and adolescents (cases) – BMI-SDS at the beginning and after the intervention (BMI-SDS reduction) for genotypes at rs17782313 and rs12970134

SNP	BMI-SDS[1]	Genotype	n (%)	Mean ± SD	Estimate	95% CI	p value[2]
Beginning of the intervention							
rs17782313	all subjects	CC	43 (11.72)	2.39 ± 0.46	0.044	–0.030 to 0.119	0.244
		CT	149 (40.60)	2.45 ± 0.50			
		TT	175 (47.68)	2.36 ± 0.50			
	females	CC	20 (9.76)	2.28 ± 0.49	0.009	–0.098 to 0.117	0.866
		CT	87 (42.44)	2.45 ± 0.52			
		TT	98 (47.8)	2.36 ± 0.51			
	males	CC	23 (14.2)	2.49 ± 0.43	0.079	–0.016 to 0.174	0.107
		CT	62 (38.27)	2.45 ± 0.48			
		TT	77 (47.53)	2.36 ± 0.50			
rs12970134	all subjects	AA	52 (14.17)	2.39 ± 0.48	0.057	–0.015 to 0.129	0.124
		AG	149 (40.60)	2.47 ± 0.51			
		GG	166 (45.23)	2.34 ± 0.49			
	females	AA	27 (13.17)	2.27 ± 0.48	0.020	–0.081 to 0.122	0.695
		AG	85 (41.46)	2.49 ± 0.53			
		GG	93 (45.37)	2.33 ± 0.50			
	males	AA	52 (14.17)	2.39 ± 0.48	0.103	0.010–0.197	0.032
		AG	149 (40.60)	2.47 ± 0.51			
		GG	166 (45.23)	2.34 ± 0.49			
After the intervention (BMI-SDS reduction)							
rs17782313	all subjects	CC	43 (11.72)	0.29 ± 0.37	0.035	–0.014 to 0.084	0.163
		CT	149 (40.60)	0.23 ± 0.35			
		TT	175 (47.68)	0.22 ± 0.30			
	females	CC	20 (9.76)	0.44 ± 0.38	0.086	0.013–0.159	0.021
		CT	87 (42.44)	0.21 ± 0.36			
		TT	98 (47.8)	0.19 ± 0.19			
	males	CC	23 (14.2)	0.17 ± 0.31	–0.016	–0.079 to 0.046	0.547
		CT	62 (38,271)	0.27 ± 0.33			
		TT	77 (47.53)	0.25 ± 0.24			
rs12970134	all subjects	AA	52 (14.17)	0.32 ± 0.38	0.039	–0.008 to 0.087	0.102
		AG	149 (40.60)	0.22 ± 0.34			
		GG	166 (45.23)	0.22 ± 0.30			
	females	AA	27 (13.17)	0.44 ± 0.39	0.092	0.024–0.160	0.009
		AG	85 (41.46)	0.19 ± 0.35			
		GG	93 (45.37)	0.19 ± 0.34			
	males	AA	25 (15.43)	0.20 ± 0.33	–0.019	–0.081 to 0.043	0.614
		AG	64 (39.51)	0.24 ± 0.33			
		GG	73 (45.06)	0.26 ± 0.23			

[1]Effect of one copy of the minor (risk) allele in the additive genetic model as determined by linear regression adjusted for age (or age and sex in the analysis of both sexes) in the beginning of the intervention.
[2]Positive values indicate relative weight reduction in units of BMI-SDS after the intervention.
[3]Effect for one copy of the minor (risk) allele in the additive genetic model as determined by linear regression adjusted for age and BMI-SDS at the beginning of the intervention program (or age, BMI-SDS at the beginning of the intervention program and sex in the analysis of both sexes)
[4]Two-sided p value.

creased diastolic blood pressure. Males with the risk genotype had higher diastolic blood pressure, whereas the opposite was found in females. It has recently been suggested that the central melanocortinergic tone significantly influences blood pressure in humans [25]. In addition, obese individuals with functionally relevant *MC4R* mutations showed lower rates of hypertension than controls [25] (46 carriers of functionally relevant *MC4R* mutations) as well as lower diastolic blood pressure [26] (8 carriers of functionally relevant *MC4R* mutations). Gender interaction was not analyzed in these studies [25, 26], probably due to the small sample sizes. However, with regard to the rs17782313 genotype, Timpson et al. [27] did not find evidence for an association with blood pressure – but unlike our study they did not address this question in sex-

stratified analyses. Additionally, we did not observe evidence for association(s) of the risk variants of rs17782313 (and rs12970134) with BMI-SDS, waist circumference, weight, height, cholesterol, triglycerides and glucose when stratifying for sex. Association of the obesity risk variant at rs17782313 with body height was originally only described in adults but not in children [4], thus confirming our data.

Lastly, we analyzed the impact of the *MC4R* risk alleles in 367 overweight and obese children and adolescents who participated in a weight management lifestyle intervention program – again focusing on sex-related effects. In contrast to the general effect of the risk-allele C for obesity we observed that female carriers of at least one copy of the obesity-risk allele had a greater BMI-SDS reduction during the 1-year intervention than female non-carriers. Of course, this explorative finding requires to be replicated in larger and independent study groups participating in a similarly designed intervention. In the literature and beyond sex-specific effects, Haupt et al. [22] reported that rs17782313 had no impact on changes in body weight or fat distribution as assessed in 242 non-diabetic German adults who participated in a 9-month adult lifestyle intervention program. Moreover, two previous studies have described a lack of association with weight reduction in carriers of functionally relevant *MC4R* mutations [21, 28]. This lack of association, however, is possibly due to the very small number of analyzed mutation carriers (9 in [21] and 4 in [28]). Beyond these findings, our study also has limitations. First of all analyses were performed in a moderately sized sample which is underpowered to detect moderate or small effects, underlining the necessity to conduct larger studies with a focus on sex-specific effects. Secondly, with regard to the multiple phenotypes analyzed, we deem it important to perform explorative analyses on a variety of phenotypes to counteract biased reporting. Thirdly, the quantitative trait analyses were performed in a selected sample of cases (overweight and obese children and adolescents) instead of a population-based sample. This constraint may also be a strength of our study as we might have detected effects more specific for early onset obesity which might be overlooked in population-based samples.

Conclusions

In conclusion, we confirmed the association of rs17782313 and rs12970134 near the *MC4R* with early onset obesity, but found no sex-specific effects. Increased insulin levels were observed among obese cases with obesity risk alleles, again a result not related to sex. We observed exploratory evidence for sex-specific effects for diastolic blood pressure and BMI-SDS reduction after a 1-year lifestyle intervention to reduce weight.

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Disclosure Statement

The authors declare no conflicts of interest.

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