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ORIGINAL ARTICLE

Strong influence of variants near *MC4R* on adiposity in children and adults: a cross-sectional study in Indian population

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Common variants near melanocortin 4 receptor (*MC4R*) gene are shown to be associated with adiposity but have varied effects in different age groups. Among Indians, studies have shown association of these variants with obesity in adults, but their association in children is yet to be confirmed. We evaluated association of rs17782313 and rs12970134 near *MC4R* with adiposity and related traits in Indians including 1362 children and 4077 adults (consisting of 2049 diabetic and 2028 nondiabetic adult subjects). Both variants rs17782313 and rs12970134 showed strong association with adiposity measures (weight, body mass index and waist circumference) in children (*P*-range 7.6×10^{-5}–3.8×10^{-12}) and nominal association in nondiabetic adults (*P*-range 0.05–0.003). Effect sizes on adiposity measures in children (β range 0.22–0.26 *Z*-score) were ~3-fold higher compared with adults (β range 0.06–0.08). The minor alleles of both variants showed borderline association (*P*-range 0.08–0.04) with risk of type 2 diabetes in adults. Meta-analysis of rs12970134 in >12 000 Indian adults corroborated its association with adiposity ($P \leqslant 2.2 \times 10^{-9}$), homeostasis model assessment-estimated insulin resistance ($P = 4.0 \times 10^{-5}$) and type 2 diabetes ($P = 0.003$) with only moderate heterogeneity, suggesting similar effect on adult Indians residing in different geographical regions. In conclusion, the study demonstrates association of variants near *MC4R* with obesity and related traits in Indian children and adults, with higher impact during childhood.
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INTRODUCTION

Melanocortin 4 receptor (MC4R) has an important role in regulation of energy homeostasis. Mutations in *MC4R* have been known as the most common cause of monogenic obesity for several years.[1] In recent times, genome-wide association studies have also implicated common variants near *MC4R* in obesity at population level.[2,3] Loos *et al.*[2] found association of rs17782313 near *MC4R* with body mass index (BMI) in European adults and children. Chambers *et al.*[3] demonstrated association of rs12970134 near *MC4R* with waist circumference (WC) and insulin resistance in Indian adults living in UK. Further replication studies in different population robustly confirmed the association of both variants with obesity.[1]

Variants near *MC4R* (rs17782313 and rs12970134) have been shown to have age-dependent effect on adiposity, showing stronger association during childhood than later in life.[2,4,5] This demands further investigation of these variants in different age groups from various ethnic groups to capture the whole spectrum of their effect. The risk alleles of both the variants are more prevalent in Indians (~36–40%)[3,6–8] compared with Europeans (~27–31%), Asians (~18–24%) and Africans (~13–31%) (HapMap release no. 27), suggesting higher contribution to susceptibility towards obesity in Indians. Previous studies have confirmed association of variants near *MC4R* with obesity in adult Indians;[6–9] however, their association in children is yet to be confirmed. There is only one recent study by Vasan *et al.*[10] that investigated association of rs17782313 with obesity in younger ages but failed to find any association.

Here we investigated association of two widely studied variants near *MC4R*-rs17782313 and rs12970134 with obesity and related traits in 1362 Indian children and 4077 adults (consisting of 2049 diabetic and 2028 nondiabetic subjects) and compared the effect sizes in two age groups. We also performed meta-analysis on adult Indians using data from previous studies.[3,6,7] Previous studies in Indian population

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have also demonstrated influence of variants near *MC4R* on insulin resistance and risk of type 2 diabetes.[3,7] Therefore, we sought to examine the association of variants near *MC4R* with type 2 diabetes in North Indian adult subjects and further performed meta-analysis with previous studies to determine their impact on risk of type 2 diabetes in adult Indians. Our study would give comprehensive insight into influence of these variants in genetic etiology of obesity and related metabolic traits in high-risk Indian population.

MATERIALS AND METHODS

The study involved participation of 5439 unrelated Indian subjects comprising of 1362 school-going children and 4077 adults. Children (aged 11–17 years) were recruited from public and private schools located in four different geographical regions of Delhi, India, as a part of health survey.[11] Adult population included 2049 type 2 diabetes patients and 2028 nondiabetic subjects, recruited from urban regions in and around Delhi as a part of an ongoing type 2 diabetes case–control study.[12] The type 2 diabetic patients were diagnosed as per World Health Organization criteria.[13] Briefly, individuals of at least 30 years of age having fasting glucose levels $\geqslant 7.0\,\mathrm{mmol\,l^{-1}}$ and/or postprandial glucose levels $\geqslant 11.1\,\mathrm{mmol\,l^{-1}}$ or on antidiabetic medication were included as type 2 diabetic patients. Pregnant women and type 1 diabetic patients were excluded. Nondiabetic subjects were $\geqslant 40$ years of age without family history of diabetes who had glycated hemoglobin level $\leqslant 6.0\%$ and fasting glucose level $< 6.1\,\mathrm{mmol\,l^{-1}}$. Informed written consent was obtained from all adult participants. In case of children, prior informed written consent from school authorities, parents/guardians and verbal consent from children themselves was obtained. The study was approved by ethic committees of participating institutes and was conducted according to the principles of Helsinki Declaration.

For case–control analysis of obesity, subjects were classified as normal-weight, overweight/obese (including overweight and obese subjects) and obese (only obese subjects that were subgroup of overweight/obese group). Children were classified as normal-weight ($N = 927$), overweight/obese ($N = 435$) and obese ($N = 159$) using age- and sex-specific BMI cutoffs provided by Cole *et al.*[14] Only nondiabetic adults ($N = 2028$) were considered for association analysis with obesity and quantitative traits in adults. Of the nondiabetic adults, 720 were normal-weight (BMI $< 23\,\mathrm{kg\,m^{-2}}$), 1308 were overweight/obese ($\geqslant 23\,\mathrm{kg\,m^{-2}}$) and 937 were obese ($\geqslant 25\,\mathrm{kg\,m^{-2}}$).[15]

All the recruited subjects underwent anthropometric and biochemical characterization as described previously.[12,16] Anthropometric measurements included height, weight, WC and hip circumference that were taken using the standard methods. Biochemical measurements including levels of fasting plasma glucose, fasting plasma insulin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride and calculation of homeostasis model assessment-estimated insulin resistance (HOMA-IR) were performed as discussed earlier.[12,16] Anthropometric and clinical characteristics of study populations are summarized in Table 1, whereas detailed description of characteristics in different study groups is provided in Supplementary Tables S1 and S2. Genotyping was performed using iPLEX assay (Sequenom, San Diego, CA, USA). The average call rate for genotyping was 95% with >97% concordance in 225 duplicates (Supplementary Table S3). Genotype distributions for both single-nucleotide polymorphisms were in Hardy–Weinberg equilibrium ($P \geqslant 0.05$).

Statistical analyses were performed using PLINK v. 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) and SPSS v. 17.0 (SPSS, Chicago, IL, USA). Hardy–Weinberg equilibrium in genotype distribution was checked using χ^2-test. Continuous variables were transformed to normal distribution using inverse normal transformation, separately in children and nondiabetic adults. We assessed association of variants with categorical and continuous traits using logistic and linear regression, respectively, assuming additive models adjusted for age and sex (BMI as appropriate). Effect sizes and odds ratios (ORs) are presented with respect to minor allele. Linkage disequilibrium between the single-nucleotide polymorphisms was determined using Haploview 4.1.[17] We performed 10 000 permutations to account for multiple testing. Statistical power was calculated using Quanto (http://hydra.usc.edu/gxe/). Our study was well powered (>85%) to detect the association of variants with obesity in children and adults (Supplementary Table S4).

To comprehensively evaluate the impact of variants near *MC4R* on obesity, type 2 diabetes and related traits in Indian adults, we performed meta-analysis by combining additive summary statistic data of obesity parameters (per allele effect size of BMI, WC and weight), insulin resistance (per allele effect size of HOMA-IR) and risk of type 2 diabetes (OR) of variant rs12970134 from previous studies on Indian population and present study. We searched PubMed literature (till December 2011) using search terms 'MC4R', 'MC4R association in Indian', 'MC4R and obesity in Indian' and 'MC4R and diabetes in Indian' to catalog all previous studies that have examined association of *MC4R* with obesity and related traits in Indian population. We identified five relevant publications that had performed association analysis of *MC4R* rs12970134 with obesity or related traits in Indian population. The details of the studies and characteristics of subjects of each study included in the meta-analysis are provided in Supplementary Table S5. Of these five previous publications, two studies (Taylor *et al.*[8] and Dorajoo *et al.*[9]) could not be meta-analyzed as their summary statistic data (*Z*-score unit) were not compatible with other three studies.[3,6,7] Thus, the meta-analysis for obesity traits (BMI, WC and weight) included three previous studies (Chambers *et al.*,[3] Been *et al.*[6] and Janipalli *et al.*[7]) and the present study. Meta-analysis for insulin resistance included summary statistic data (β for HOMA-IR) from Chambers *et al.*[3] and

Table 1 Descriptive characteristics of study population

Trait	*Children*	*Type 2 diabetes patients*	*Nondiabetic subjects*
N (M/F)	1362 (620/742)	2049 (1202/847)	2028 (1111/917)
Age (years)	13.96 (1.81)	54.57 (10.50)	53.65 (10.60)
Weight (kg)	50.35 (14.95)	67.56 (11.71)	63.69 (13.16)
Height (m)	1.55 (0.10)	1.62 (0.09)	1.60 (0.09)
BMI ($\mathrm{kg\,m^{-2}}$)	20.64 (5.07)	25.91 (4.43)	24.92 (4.89)
WC (cm)	72.37 (11.96)	92.99 (11.14)	89.00 (11.67)
WHR	0.84 (0.07)	0.98 (0.07)	0.92 (0.08)
Total cholesterol ($\mathrm{mmol\,l^{-1}}$)	3.75 (0.88)	4.56 (1.19)	4.71 (1.07)
HDL-C ($\mathrm{mmol\,l^{-1}}$)	1.13 (0.20)	1.14 (0.31)	1.20 (0.33)
LDL-C ($\mathrm{mmol\,l^{-1}}$)	2.26 (0.66)	2.76 (0.96)	2.98 (0.84)
Triglycerides ($\mathrm{mmol\,l^{-1}}$)	1.26 (0.47)	1.77 (1.16)	1.43 (0.75)
FPG ($\mathrm{mmol\,l^{-1}}$)	5.00 (0.58)	8.72 (3.54)	4.80 (0.63)
FPI ($\mathrm{pmol\,l^{-1}}$)	55.97 (40.66)	—	48.19 (40.01)
HOMA-IR	2.07 (1.54)	—	1.75 (1.53)

Abbreviations: F, females; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; M, males; N, number of subjects; WC, waist circumference; WHR, waist-hip ratio.
Data are presented as mean with s.d. in parentheses.

present study, whereas meta-analysis for type 2 diabetes included summary statistics (OR for risk of type 2 diabetes) from Chambers *et al.*,[3] Janipalli *et al.*[7] and present study. After abstracting the relevant data from all the studies, we performed meta-analysis using fixed effect model. Heterogeneity in effect sizes across the studies was tested using Cochran's Q statistics as implicated in PLINK v. 1.07.

RESULTS

Both rs17782313 and rs12970134, that were in linkage disequilibrium ($r^2 = 0.88$), were associated with increased risk of being overweight/obese (OR = 1.67, $P = 1.3 \times 10^{-9}$ and OR = 1.60, $P = 2.0 \times 10^{-8}$, respectively) and obese (OR = 1.73, $P = 6.9 \times 10^{-6}$ and OR = 1.62, $P = 7.6 \times 10^{-5}$, respectively) in children (Table 2). Association analysis with measures of adiposity in children showed association of rs17782313 and rs12970134 with BMI ($\beta = 0.24$ *Z*-score units, $P = 8.5 \times 10^{-11}$ and $\beta = 0.22$, $P = 6.7 \times 10^{-9}$, respectively), weight ($\beta = 0.24$, $P = 3.5 \times 10^{-11}$ and $\beta = 0.22$, $P = 7.4 \times 10^{-10}$, respectively) and WC ($\beta = 0.26$, $P = 3.8 \times 10^{-12}$ and $\beta = 0.24$, $P = 4.3 \times 10^{-10}$, respectively). In children, rs12970134 also showed BMI-independent association with fasting plasma insulin levels and HOMA-IR index ($P = 0.006$ and 0.005, respectively) (Table 2).

In nondiabetic adults, only nominal association of rs17782313 and rs12970134 with risk of overweight/obesity (BMI $\geqslant 23\,kg\,m^{-2}$) (OR = 1.18, $P = 0.028$ and OR = 1.17, $P = 0.028$, respectively) and relatively stronger association with obesity (BMI $\geqslant 25\,kg\,m^{-2}$) (OR = 1.27, $P = 0.003$ and OR = 1.24, $P = 0.005$, respectively) was found (Table 2). We also observed nominal association of rs17782313 and rs12970134 with BMI ($\beta = 0.08$, $P = 0.027$ and $\beta = 0.08$, $P = 0.018$, respectively), weight ($\beta = 0.07$, $P = 0.036$ and $\beta = 0.07$, $P = 0.025$, respectively) and WC ($\beta = 0.07$, $P = 0.034$ and $\beta = 0.06$, $P = 0.05$), respectively. Meta-analysis involving >12 000 adult Indians provided stronger evidence for association of rs12970134 with BMI, WC, weight and HOMA-IR ($P \leqslant 4.0 \times 10^{-5}$) (Table 3).

Next, we compared the impact of the variants near *MC4R* on adiposity measures in children and adults to determine their age-specific effect. The effect sizes of the variants on weight, WC and BMI were significantly different between children and adults (*Q*-range $0.002–6.0 \times 10^{-4}$; I^2 range 87.4–92.9) (Table 2), with higher impact on children compared with adults.

Association analysis with type 2 diabetes showed borderline association of rs17782313 ($P = 0.04$) and rs12970134 ($P = 0.08$) (Table 2). Meta-analysis of rs12970134 with the previous studies further affirmed the association with type 2 diabetes ($P = 0.003$). No heterogeneity in the effect sizes of rs17782313 for type 2 diabetes across the studies was observed ($P_{het} = 0.79$; $I^2 = 0.0\%$) (Table 3). Despite the association of the variants with obesity traits and type 2 diabetes, these variants were not found to be associated with fasting plasma glucose levels and lipid profile, either in children or in adults (Supplementary Table S6).

DISCUSSION

Understanding of genetic factors involved in predisposition to obesity in Indians is limited, particularly in context of childhood obesity. Only recent studies from our group provided evidence for association of common variants with obesity in Indian children.[16,18] To get further insight, here we evaluated association of variants near *MC4R* with obesity and related traits in Indian children (11–17 years) and adults (>40 years) and compared their effect sizes on adiposity in these two study population of different age groups. The present study establishes strong association of variants rs17782313 and rs12970134 near *MC4R* with adiposity measures in Indian children for the first time and confirms their association with adiposity measures and type 2 diabetes in adult Indians. The findings provide evidence for stronger influence of variants near *MC4R* on adiposity measures in Indian children.

Association of rs17782313 and rs12970134 with adiposity measures suggested that these variants might mediate susceptibility to obesity by influencing the overall body size. Children who were homozygote for minor allele of rs17782313 had ~7.5 kg increased weight, $\sim 2.5\,kg\,m^{-2}$ increased BMI and ~6.4 cm increased WC compared with homozygotes for other allele. Consistently in adults, individuals homozygous for minor allele of rs17782313 had $\sim 0.8\,kg\,m^{-2}$ increased BMI, ~1.5 cm increased WC and ~2.0 kg increased weight compared with homozygotes for other allele. We also confirmed association of rs12970134 with HOMA-IR and fasting plasma insulin levels independent of BMI in children. Thus, our study supports the observation of pleiotropic effect of these common variants on weight regulation, linear growth and insulin levels. Previous studies have also suggested nominal association of variants near *MC4R* with levels of high-density lipoprotein cholesterol, total cholesterol and triglyceride.[3,7] We did observe BMI-dependent association of both the variants with low-density lipoprotein cholesterol ($P < 0.004$), triglyceride ($P < 0.008$) and total cholesterol ($P < 0.008$) in children. However, associations were vanished after adjusting for BMI, indicating that the effect of variants near *MC4R* on lipid profile is mediated through BMI in Indian population.

We observed strong age-dependent influence of both variants on obesity parameters, with effect sizes ~3-fold higher in children compared with adults. Moreover, variant rs17782313 conferred higher risk of obesity in Indians compared with Europeans, particularly in children. To the best of our knowledge, the effect size of variants (rs17782313 and rs12970134) near *MC4R* on adiposity traits in Indian children is highest compared with effect sizes from other populations reported so far.[1] The observed OR for risk of obesity in Indian children for rs17782313 (OR = 1.73) was higher compared with previous reports in European children (OR range 1.20–1.40), even though mean BMI was similar in obese category in both studies ($29.5–33.0\,kg\,m^{-2}$ in European vs $30.14\,kg\,m^{-2}$ in present study).[2] Indian children with risk allele for rs17782313 have two-fold higher increase in BMI (0.24 *Z*-score) compared with European children (0.01–0.13 *Z*-score).[2,19] Environmental factors including diet and physical activity have been suggested to have important role in modulating effect of genetic variants.[20,21] The observed differences in effect sizes of variants near *MC4R* on adiposity measures in children might be due to modulation by population-specific environmental factors, which may have strong influence during childhood and adolescence. However, further well-designed studies are required in this direction to confirm influence of environmental factors in modulation of effect of variants near *MC4R* in Indian specifically in children.

In adults, nominal association of rs17782313 and rs12970134 with risk of overweight and obesity was found in our study population; however, meta-analysis involving >12 000 adult Indians provided stronger evidence for association of rs12970134 with BMI, WC, weight and HOMA-IR. Interestingly, studies included in meta-analysis differ in many aspects including geographical locations and study design (Supplementary Table S5). Chambers *et al.*[3] included population samples, whereas other studies involved case–control subjects. Moreover, Chambers *et al.*[3] included migrant Indians, whereas Janipalli *et al.*[7] study had Indian residents from rural setting, and subjects in Bean *et al.*[6] study belonged to Sikh community from North India. The anthropometric characteristics of subjects across the studies also vary. Despite these differences, only

Table 2 Association of MC4R variants with obesity and related traits in children and adults in Indians

	Children (N = 1362)					*Adults (N = 2028)*[a]					*Heterogeneity*[b]	
	n *(frequency)*					n *(frequency)*						
Trait												
rs17782313	*CC*	*CT*	*TT*	*OR (95% CI)*	P	*CC*	*CT*	*TT*	*OR (95% CI)*	P	Q	I^2
Obesity												
Normal-weight subjects	118 (0.13)	380 (0.42)	406 (0.45)	1.00		85 (0.13)	290 (0.45)	268 (0.42)	1.00			
Overweight/obese subjects	94 (0.22)	209 (0.49)	122 (0.29)	1.67 (1.42–1.97)	$\mathbf{1.3 \times 10^{-9}}$	158 (0.15)	527 (0.50)	375 (0.35)	1.18 (1.02–1.36)	0.028	0.002	89.6
Obese subjects	36 (0.23)	78 (0.50)	43 (0.27)	1.73 (1.36–2.19)	$\mathbf{6.9 \times 10^{-6}}$	124 (0.16)	377 (0.50)	255 (0.34)	1.27 (1.09–1.48)	**0.003**	0.031	78.0
T2D												
Nondiabetic subjects						245 (0.14)	819 (0.48)	649 (0.38)	1.00			
Diabetic patients						309 (0.17)	896 (0.48)	658 (0.35)	1.11 (1.00–1.22)	0.041		
Quantitative traits	*Mean (s.d.)*			*β (95% CI)*	P	*Mean (s.d.)*			*β (95% CI)*			
Genotype frequency	0.16	0.44	0.40			0.14	0.48	0.38				
Z-BMI	0.28 (0.95)	0.07 (1.00)	−0.19 (0.96)	0.24 (0.17–0.32)	$\mathbf{8.5 \times 10^{-11}}$	0.05 (0.99)	−0.02 (0.98)	−0.12 (0.99)	0.08 (0.01–0.14)	**0.027**	0.001	90.8
Z-WC	0.26 (0.96)	0.10 (1.01)	−0.23 (0.93)	0.26 (0.19–0.34)	$\mathbf{3.8 \times 10^{-12}}$	0.04 (0.98)	−0.02 (0.96)	−0.10 (0.98)	0.07 (0.01–0.14)	0.034	2.0×10^{-4}	92.9
Z-Weight	0.28 (0.99)	0.06 (1.00)	−0.18 (0.95)	0.24 (0.17–0.31)	$\mathbf{3.5 \times 10^{-11}}$	0.06 (1.00)	−0.04 (0.95)	−0.08 (0.99)	0.07 (0.00–0.14)	0.036	6.0×10^{-4}	91.4
Z-Height	0.11 (1.02)	0.00 (1.00)	−0.06 (0.96)	0.09 (0.02–0.15)	**0.008**	0.03 (0.95)	−0.01 (0.99)	0.02 (0.98)	0.03 (−0.02 to 0.08)	0.244	0.16	49.8
Z-WHR	0.07 (0.96)	0.11 (1.00)	−0.15 (0.96)	0.13 (0.06–0.20)	$\mathbf{2.0 \times 10^{-4}}$	0.05 (0.98)	−0.02 (0.98)	0.00 (0.96)	0.03 (−0.02 to 0.09)	0.262	0.028	79.2
Z-Insulin	0.21 (1.04)	0.06 (0.96)	−0.17 (0.99)	0.07 (0.01–0.14)	0.032	0.00 (0.97)	−0.02 (1.00)	−0.10 (1.00)	0.03 (−0.04 to 0.10)	0.379	0.39	0.0
Z-HOMA-IR	0.22 (1.02)	0.07 (0.97)	−0.18 (0.97)	0.08 (0.01–0.15)	**0.020**	−0.01 (0.97)	−0.04 (1.01)	−0.10 (1.00)	0.02 (−0.05 to 0.09)	0.513	0.24	26.9
	n *(frequency)*					n *(frequency)*						
rs12970134	*AA*	*AG*	*GG*	*OR (95% CI)*	P	*AA*	*AG*	*GG*	*OR (95% CI)*	P	Q	I^2
Obesity												
Normal-weight subjects	118 (0.13)	379 (0.42)	411 (0.45)	1.00		85 (0.12)	302 (0.44)	305 (0.44)	1.00			
Overweight/obese subjects	93 (0.22)	203 (0.47)	131 (0.31)	1.60 (1.36–1.89)	$\mathbf{2.0 \times 10^{-8}}$	165 (0.13)	608 (0.49)	467 (0.38)	1.17 (1.02–1.34)	0.028	0.004	87.7
Obese subjects	34 (0.22)	76 (0.48)	47 (0.30)	1.62 (1.27–2.05)	$\mathbf{7.6 \times 10^{-5}}$	130 (0.15)	438 (0.49)	326 (0.36)	1.24 (1.07–1.44)	**0.005**	0.06	71.5
T2D												
Nondiabetic subjects						251 (0.13)	911 (0.47)	779 (0.40)	1.00			
Diabetic patients						302 (0.15)	889 (0.46)	756 (0.39)	1.09 (0.99–1.19)	0.080		
Quantitative traits	*Mean (s.d.)*			*β (95% CI)*	P	*Mean (s.d.)*			*β (95% CI)*			
Genotype frequency	0.16	0.44	0.40			0.13	0.47	0.40				
Z-BMI	0.24 (0.94)	0.06 (1.01)	−0.16 (0.97)	0.22 (0.14–0.29)	$\mathbf{6.7 \times 10^{-9}}$	0.08 (0.99)	0.04 (0.99)	−0.07 (0.99)	0.08 (0.01–0.14)	**0.018**	0.005	87.4
Z-WC	0.22 (0.94)	0.10 (1.02)	−0.20 (0.94)	0.24 (0.16–0.31)	$\mathbf{4.3 \times 10^{-10}}$	0.04 (0.99)	0.03 (0.99)	−0.06 (0.98)	0.06 (0.00–0.13)	0.050	6.0×10^{-4}	91.6
Z-Weight	0.24 (0.98)	0.06 (1.01)	−0.17 (0.95)	0.22 (0.15–0.29)	$\mathbf{7.4 \times 10^{-10}}$	0.06 (1.00)	0.02 (1.00)	−0.05 (0.97)	0.07 (0.01–0.14)	**0.025**	0.002	89.4
Z-Height	0.10 (1.01)	0.01 (1.00)	−0.07 (0.96)	0.09 (0.03–0.15)	**0.004**	-0.02 (0.96)	0.00 (1.00)	0.00 (0.98)	0.03 (−0.01 to 0.08)	0.178	0.13	56.6
Z-WHR	0.08 (0.94)	0.09 (1.00)	−0.13 (0.98)	0.11 (0.04–0.18)	**0.002**	0.05 (0.98)	−0.02 (1.00)	0.00 (0.98)	0.04 (−0.01 to 0.09)	0.135	0.11	60.4
Z-Insulin	0.25 (1.02)	0.05 (0.95)	−0.17 (1.00)	0.09 (0.03–0.16)	**0.006**	0.03 (0.95)	0.03 (0.98)	−0.03 (0.99)	0.01 (−0.06 to 0.07)	0.836	0.06	71.1
Z-HOMA-IR	0.26 (1.01)	0.05 (0.95)	−0.17 (0.99)	0.10 (0.03–0.16)	**0.005**	0.04 (0.96)	0.01 (0.99)	−0.03 (0.99)	0.00 (−0.06 to 0.07)	0.934	0.05	74.4

Abbreviations: CI, confidence interval; I^2, I^2 heterogeneity index (0–100); N, total number of subjects in the study; n, number of subjects with genotype data; OR, odds ratio with respect to minor allele; *Q*, *P*-value for Cochrane's Q statistic for heterogeneity of effects.

β values represent change in *Z*-score per minor allele. Obese category is subgroup of overweight per obese group. Overweight per obese category in adults includes subjects with BMI $\geqslant 23\,kg\,m^{-2}$, whereas in children, BMI cutoffs corresponding to $25\,kg\,m^{-2}$ for adults as provided by Cole *et al.*[14] Obese category in adults includes subjects with BMI $\geqslant 25\,kg\,m^{-2}$, whereas in children, BMI cutoffs corresponding to $30\,kg\,m^{-2}$ for adults as provided by Cole *et al.* Analyses were adjusted for age and sex for all the traits except for insulin and HOMA-IR that are adjusted for age, sex and BMI.

[a]Only nondiabetic subjects were considered for association analysis in adults except for case–control analysis for T2D.

[b]Comparison of effect sizes of *MC4R* variants on measures of adiposity between children and adults. *P*-values in bold remained significant after 10 000 permutations. Please note that number of subjects (*n*) in different categories of obesity status do not add up to total subjects (*N*) because of missing genotypes.

Table 3 Meta-analysis of rs12970134 in adult Indians

			Meta-analysis			
Trait	*Study (reference)*	*Sample size*	*β/OR (95% CI)*	P	I^2	Q
BMI ($kg\,m^{-2}$)	Present study	1927	0.38 (0.06–0.69)			
	Chambers *et al.*[3a]	7394	0.25 (0.10–0.39)			
	Been *et al.*[6]	1491	0.67 (0.30–1.04)			
	Janipalli *et al.*[7]	1306	0.43 (0.19–0.66)			
	Meta-analysis	12 118	0.35 (0.24–0.46)	2.2×10^{-9}	38.51	0.18
WC (cm)	Present study	1914	0.75 (0.00–1.50)			
	Chambers *et al.*[3a]	11 955	0.74 (0.42–1.07)			
	Been *et al.*[6]	1491	1.76 (0.68–2.84)			
	Janipalli *et al.*[7]	1306	1.06 (0.38–1.74)			
	Meta-analysis	16 666	0.85 (0.59–1.10)	1.5×10^{-10}	17.54	0.30
Weight (kg)	Present study	1928	0.98 (0.12–1.85)			
	Chambers *et al.*[3a]	7394	0.82 (0.40–1.24)			
	Been *et al.*[6]	1491	1.76 (0.68–2.84)			
	Janipalli *et al.*[7]	1306	1.18 (0.49–1.87)			
	Meta-analysis	12 119	0.99 (0.68–1.31)	4.6×10^{-10}	0.00	0.41
HOMA-IR (%)	Present study	1739	2.95 (-2.69–8.58)			
	Chambers *et al.*[3a]	7394	5.39 (2.78–8.07)			
	Meta-analysis	9133	4.96 (2.59–7.32)	4.0×10^{-5}	0.00	0.44
Type 2 diabetes	Present study	Cases = 1941 Controls = 1947	1.08 (0.99–1.19)			
	Chambers *et al.*[3a]	Cases ~1279[b] Controls ~6115[b]	1.07 (0.98–1.17)			
	Janipalli *et al.*[7]	Cases = 1714 Controls = 1419	1.12 (1.01–1.24)			
	Meta-analysis	Cases ~4934 Controls ~9481	1.09 (1.03–1.15)	0.003	0.00	0.79

Abbreviations: β, per allele effect size with respect to minor allele A; BMI, body mass index; HOMA-IR, homeostasis model assessment-estimated insulin resistance; I^2, I^2 heterogeneity index (0–100); OR, odds ratio with respect to minor allele A; *Q*, *P*-value for Cochrane's Q statistic; WC, waist circumference.
[a]Summary statistics of Indian Asian ancestry samples in stage 2 were considered for the analysis, except for WC, which also included data from European samples in stage 2.
[b]Number of cases and controls were estimated from the proportions provided in the study.

low-to-moderate heterogeneity in effect sizes across studies were observed (*Q*-range 0.18–0.79; I^2 range = 0.0–38.51), indicating similar effect of variants near *MC4R* on adiposity in adult Indians residing in different geographical regions.

In adults, variant rs17782313 had higher effect on risk of obesity (BMI $\geqslant 25\,kg\,m^{-2}$) in Indians compared with Europeans with BMI $\geqslant 25\,kg\,m^{-2}$ (OR for Indians = 1.27 and OR for Europeans 1.08; $P_{het} = 0.047$, $I^2 = 74.5\%$).[2] But the effect sizes of rs17782313 for BMI (~ 0.25–$0.67\,kg\,m^{-2}$), WC (~ 0.74–1.76 cm) and weight (~ 0.82–1.76 kg) (Table 2) were similar in Indian and European adults (for BMI ~ 0.10–$0.30\,kg\,m^{-2}$, WC ~ 0.40–0.70 cm and weight ~ 0.70–1.00 kg).[1] The variants near *MC4R* are more prevalent in Indians compared with other populations,[3,6] and thus are likely to have higher contribution to the variance in adiposity measures in Indians.

Interestingly, a recent study on South Indian longitudinal cohort failed to detect association of variants near *MC4R* with obesity parameters during younger ages.[10] Previous studies have shown that environmental factors can modulate strength of association of common variants with obesity parameters.[20,21] The observed discrepancy in the association result might be due to the difference in study design, particularly recruitment of subjects. Vasan *et al.*[10] included subjects from rural and urban regions of South India that could have attenuated effect sizes of variants near *MC4R* on adiposity measures, resulting in lower statistical power to detect them.

Notably, because of logistic difficulty in performing pubertal staging in school premises, we could not control for it in analyses. However, the mean age at onset of puberty in India is reported to be around 12 years,[22] which suggests that majority of the children participated in the present study from the age groups of 11 to 17 years have likely begun their puberty, and we expect minimal impact on association analyses. Furthermore, in the absence of validated reference cutoffs for defining obesity in Indian children, we adopted widely used international BMI cutoffs by Cole *et al.*[14] However, we also performed association analysis using BMI cutoffs provided by a recent study in Indian children by Marwaha *et al.*,[23] which yielded similar results (data not shown).

In conclusion, we present first report of association of variants near *MC4R* with obesity in Indian children and provide further evidence for their association with adult obesity and type 2 diabetes in Indians. The study indicates that these variants have similar effect on adiposity in adults from different geographical regions, but have stronger impact on children. Given the higher influence in children, further population-based studies and functional studies would be of great importance for Indian population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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