Original Article

Combined effect of FTO and MC4R gene polymorphisms on obesity in children and adolescents in Northwest China: a case-control study

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Background and Objectives: Fat mass and obesity-associated (*FTO*) and melanocortin 4 receptor (*MC4R*) genes associated with obesity have been identified through Genome-wide Association Studies. However, no multiple loci interaction studies have been conducted in the Chinese population. This study investigated whether the combined effects of *FTO* and *MC4R* increase the risk of obesity in children and adolescents living in Northwest China. **Methods and Study Design:** A total of 370 subjects (170 overweight/obese and 200 normal BMI subjects according to the Working Group on Obesity in China criteria) were enrolled using the random sampling method. *FTO* rs9939609 and rs9935401 and *MC4R* rs12970134 and rs17782313 interactions were analysed through generalized multifactor dimensionality reduction, and logistic regression models were used to calculate the risk of the relationship between genotypes and obesity. **Results:** Generalized multifactor dimensionality reduction analysis showed a significant gene–gene interaction among *FTO* rs9939609/MC4R rs12970134/*MC4R* rs17782313, with a score of 10/10 for the cross-validation consistency and 9 for the sign test (p=0.011). A 2.453-fold increased risk of obesity was observed in individuals carrying the genotypes of *FTO* rs9939609 TA/AA, *MC4R* rs12970134 GA/AA, and *MC4R* rs17782313 TC/CC (adjusted for age, sex, and ethnicity; 95% CI=1.12–5.37, p=0.025). **Conclusions:** Our results suggested that *FTO* rs9939609, *MC4R* rs12970134, and *MC4R* rs17782313 are strongly associated with obesity. The combined effects were highly significant on obesity in children and adolescents living in Northwest China.

Key Words: childhood obesity, gene–gene interaction, *FTO*, *MC4R*, GMDR

INTRODUCTION

Obesity is a common chronic metabolic disease that is influenced by the combined effect of multiple genetic and environmental factors. In 2014, more than 1.9 billion adults (approximately 39%; age, >18 years) were overweight, and of these, over 600 million adults (approximately 13%) were obese. Notably, 41 million children (age, ≤5 years) were overweight or obese.[1] The effect of obesity on physical and psychological functions in childhood is becoming serious, and health damage is becoming more common at a younger age. Obesity in children and adolescents can lead to high blood pressure, diabetes, coronary heart disease, and other chronic non-communicable diseases in adulthood.[2] Presently, obesity-promoting patterns such as sedentary lifestyle and energy-dense palatable food choices are developing, but not everyone is turning obese because genetic susceptibilities of individuals are different.[3] The incidence of childhood obesity has a significant genetic predisposition to the basal metabolic rate, appetite, and eating behaviour. More than 600 genes, markers, and chromosomal regions are linked with human obesity phenotypes.[4] Different genetic changes can lead to fat accumulation in different regions and different obesity phenotypes. However, there is no explicit gene that results in abundant fat accumulation in vivo.[4] Moreover, obesity is likely to be a result of superimposition of the effects of different genes.[5] Thus, after the analysis of approximately 20 genes that have been studied in recent years, we observed that *FTO* and *MC4R* were associated with being overweight in children and adolescents living in Northwest China.

FTO was the first gene to be associated with common obesity through a genome-wide association study (GWAS).[6] Significant interaction exists between the *FTO* polymorphism, energy consumption, and physical activi-

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ty.[7,8] The *FTO* rs9939609 polymorphism is associated with multiple obesity-related characteristics.[9] *FTO* rs9935401 SNPs are closely related to obesity, but only few studies have been conducted in China.[10-12] In addition, *MC4R* is the second marker for common obesity as demonstrated by another GWAS.[13] More than 90 types of *MC4R* mutations in different parts of the coding region have been reported, and most of them are related to severe obesity.[14] 'A' allele of the *MC4R* rs12970134 polymorphism plays a crucial role in the occurrence of obesity.[15-17] The *MC4R* rs17782313 variation is also a risk factor for body mass gain, body fat gain, and obesity.[13] Notably, the correlation between *MC4R* rs12970134, rs17782313, and obesity is different in different regions.[18-20] Although both these genes contribute modestly to the obesity phenotype, their combined effects are yet to be proved in large general populations. Generalized multifactor dimensionality reduction (GMDR) is developed from multifactor dimensionality reduction, which is used to determine the type and method of the gene–gene interaction. This study analysed the combined effects of *FTO* rs9939609 and rs9935401 and *MC4R* rs12970134 and rs17782313 on obese children and adolescents living in Northwest China.

METHODS

Study participants

According to the economic levels, subjects (age, 7–18 years) were selected from 15 primary schools and 10 high schools in Tongxin, Xiji, Haiyuan, Guyuan, and Jingyuan counties of Ningxia, China by using stratified cluster random sampling. A total of 3431 children and adolescents were recruited for nutritional status monitoring.

A 1:1 non-matched case-control study was conducted to estimate the sample content. The specific calculation is as follows:

$$n = \bar{p}\bar{q}\,(z_\alpha + z_\beta)^2 / (p_1 - p_0)^2$$

among them: $p_1 = p_0 RR / [1 + P_0(RR - 1)]$

$$\bar{p} = 0.5 \times (p_1 + p_0)$$

$$\bar{q} = 1 - \bar{p}$$

In the formula, n is the number of pairs required for each group, p_0 is the exposure rate of the control group, p1 is the exposure rate of the case group, $\alpha = 0.05$ (bilateral), $Z_\alpha = 1.96$, $\beta = 0.10$, and $Z_\beta = 1.28$. According to the literature, $p_0 = 0.3$, the expected effect intensity RR = 2, the estimated sample content of subnumbers is M = 200 pairs. Thus, 200 subjects should be included in the case and control groups each.

We therefore selected 200 subjects for the overweight/obese group and 200 for the normal BMI group. We excluded individuals with incomplete data or those who did not cooperate. A total of 370 subjects (170 overweight/obese and 200 normal BMI subjects according to the Working Group on Obesity in China criteria) were finally enrolled in this study by using random sampling.[21] The ethics committee of the Institutional Review Board of Ningxia Medical University approved all study protocols, and all subjects provided written informed consent (No. 2014-200).

Anthropometric measurements

The subjects' body weights with very light clothing were measured to an accuracy of ±0.2 kg, and their heights were measured to an accuracy of ±0.5 cm using a height bar. BMI was computed as weight (kg) divided by squared height (m^2). Under normal breathing, chest circumference was measured to the nearest 0.1 cm around the margin of the subscapular angle through the back to the chest with subjects standing naturally. Upper arm circumference was measured around the midpoint of the upper arm. Waist circumference was determined using an inelastic measuring tape positioned in the middle point between the last costal rib and the iliac crest in a perpendicular plane, with the patient standing with feet approximately 20 cm apart. Hip circumference was measured to the nearest 0.1 cm around the thighs, at the level of the higher diameter over the buttocks, in the standing position. Blood pressure was measured thrice to the nearest 1 mmHg with the patient being in the sitting position. All the subjects were at rest for at least 30 min before measurements. Body fat content and basal metabolism were measured using the Omron HBF356 (Omron Healthcare [China] Limited) body fat device, keeping arms and body at 90° during the measurement. Blood samples were collected after 10 h of fasting from the antecubital vein between 06:00 and 08:00 a.m. The samples were centrifuged at the survey site, and labelled plasma samples were transferred to separate tubes and then immediately transferred in cold boxes filled with ice (2°C–8°C). The samples were frozen at −80°C in a refrigerator for further analysis.

Genotyping

Genomic DNA was prepared from blood leukocytes using a DNA Extractor Whole Blood Kit (Applied Xi'an GoldMag Biotechnology). A GeneAmp PCR System (Applied Biosystems Inc., Foster City, CA, USA) was used to genotype the *FTO* rs9939609 and rs9935401 and *MC4R* rs12970134 and rs17782313 using TaqMan-based assays (Applied Biosystems Inc., Foster City, CA, USA).

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Science (SPSS®, version 13.0, Chicago, IL, USA). Results were expressed as means ± standard deviations and compared using the Student t test. The genotype distribution, allele frequency, and Hardy–Weinberg equilibrium were tested using chi-squared (X2) analysis. Gene–gene interactions on the increased risk of children and adolescent obesity were assessed using GMDR, and the risk was assessed using logistic regression analysis. A nominal two-sided *p* value of less than 0.05 was used to assess significance.

RESULTS

Characteristics of common variants

The descriptive characteristics of both the groups are presented in Table 1. The overweight/obese population had, as expected, an increased BMI (24.7±3.90 kg/m^2 vs 17.8±2.47 kg/m^2), weight (61.5±16.6 kg vs 41.4±12.5 kg), waist circumference (73.4±9.68 cm vs 63.1±8.15 cm), upper arm circumference (24.9±3.69 cm vs 20.8±3.13

Table 1. Descriptive characteristics of overweight/obese and normal weight subjects

Variants	Overweight/Obesity (n=170)	Normal weight (n=200)	T-value	*p*-value
Height (cm)	156±15.6	149±16.1	4.27	<0.05
Weight (kg)	61.5±16.6	41.4±12.5	13.0	<0.05
Biceps circumference (cm)	24.9±3.69	20.8±3.13	9.58	<0.05
Chest circumference (cm)	80.5±11.6	70.8±9.44	7.44	<0.05
Waist circumference (cm)	73.4±9.68	63.1±8.15	9.24	<0.05
Hip circumference (cm)	87.7±10.9	77.8±10.4	7.28	<0.05
BMI (kg/m^2)	24.7±3.90	17.8±2.47	19.3	<0.05
Systolic blood pressure (mm Hg)	111±13.7	107±13.8	2.40	<0.05
Diastolic blood pressure (mm Hg)	72.4±8.17	70.1±9.50	1.95	>0.05
Body fat content (%)	24.2±5.22	18.6±8.40	5.70	<0.05
Basal metabolism (kcal)	1404±247	1261±227	4.78	<0.05

BMI: body mass index. BMI was calculated as body weight (kg)/height (m^2).
Data are mean±SD. All p values are two-sided, and $p<0.05$ is significant.

cm), chest circumference (80.5±11.6 cm vs 70.8±9.44 cm), and hip circumference (87.7±10.9 cm vs 77.8±10.4 cm). Regarding the metabolic parameters of the two groups, the overweight/obese participants had significantly increased systolic blood pressure (111±13.7 mmHg vs 107±13.8 mmHg), body fat content (24.2%±5.22% vs 18.6%±8.40%), and basal metabolism (1404±247 kcal vs 1261±226 kcal), whereas their diastolic blood pressure did not differ from the controls.

Comparison of genotype distributions between the overweight/obese participants and controls

The wild type, heterozygous, and mutant homozygous genotype frequencies of the genes are shown in Table 2, and both the overweight/obese and normal weight groups were in agreement with the Hardy–Weinberg equilibrium (Table 2). These indicate that the selected sample groups of the study were appropriate. Table 2 showed that the 'A' allele of *FTO* rs9939609 in two group was significantly different (OR=1.78, 95% CI=1.15–2.77, p=0.009), the 'A' allele of *FTO* rs9935401 in two group was significantly different (OR=1.67, 95% CI=1.07–2.60, p=0.02), and the 'A' allele of MC4R rs12970134 in two group was significantly different (OR=1.51, 95% CI=1.06–2.16, p=0.02). For *MC4R* rs17782313, no statistical significance was observed.

Gene–gene interactions in the overweight/obese participants and controls

In the present study, significant high-order interactions were detected using GMDR (Table 3). With age, sex, and ethnicity adjustments, the best model included *FTO* rs9939609, *MC4R* rs12970134, and *MC4R* rs17782313, with a score of 10/10 for the cross-validation consistency and 9 for the sign test (p=0.011). In addition, the best one-locus model was *MC4R* rs12970134; it scored 5/10 for the cross-validation consistency and 6 for the sign test (p=0.377), suggesting that the contribution to obesity risk was due to the interaction of the two genes but not the additive effects of these loci. The best model for obesity

Table 2. Comparison of genotype distributions between overweight/obese and normal weight subjects

		Hardy-Weinberg equilibrium				Minor allele frequency (%)		Univariate analysis	
		Case		Control					
SNP	Allele	GENO	*p*	GENO	*p*	Case	Control	OR (95% CI)	*p*
FTO rs9939609	T > A	2/51/117	0.166	2/35/163	0.937	0.16	0.0975	1.79 (1.16-2.77)	0.009
FTO rs9935401	G > A	2/48/120	0.242	2/35/163	0.937	0.15	0.0975	1.67 (1.07-2.60)	0.022
MC4R rs12970134	G > A	8/69/93	0.283	7/58/135	0.803	0.25	0.180	1.52 (1.07-2.16)	0.020
MC4R rs17782313	T > C	8/77/85	0.069	11/64/125	0.462	0.27	0.215	1.38 (0.98-1.93)	0.064

FTO: fat mass and obesity-associated gene; MC4R: melanocortin 4 receptor gene; SNP: Single nucleotide polymorphism; CI: confidence interval; OR: odds ratio.

Table 3. GMDR analysis of gene–gene interactions in overweight/obese and normal weight subjects

Model dimension†	Optimal combination	Training balanced accuracy	Testing balanced accuracy	Cross-validation consistency	Sign test (P)
1	X3	0.571	0.508	5/10	6 (0.377)
2	X1X3	0.623	0.609	9/10	9 (0.011)
3	X1X3X4	0.627	0.602	10/10	9 (0.011)

†X1 represent *FTO* rs9939609, X2 represent *FTO* rs9935401, X3 represent *MC4R* rs12970134, and X4 represent *MC4R* rs17782313.

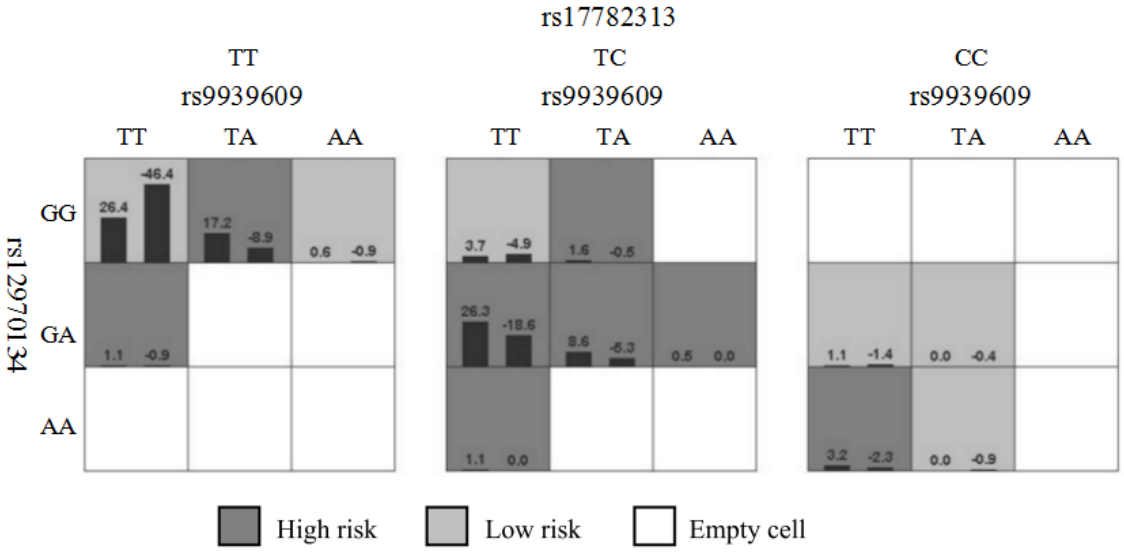

Figure 1. Best model for high risk and low risk exposure. The left side of the each small cell represents the positive score, the right side of the band represents the negative score, dark gray cells represent the high risk, the light gray cell represents the low risk, empty cell represents no combination data. For rs17782313, C is mutant allele. For rs9939609, A is mutant allele. For rs12970134, A is mutant allele.

Table 4. Risk estimation of FTO and MC4R interactions among the three-factor model

FTO rs9939609	*MC4R* rs12970134	*MC4R* rs17782313	*p*-value	OR (95%CI)
TT	GG	TT	0.000	0.495
TT	GG	TC+CC	0.625	1.29 (0.47～3.52)
TT	GA+AA	TT	0.488	2.02 (0.276～14.8)
TT	GA+AA	TC+CC	0.001	2.39 (1.44～3.98)
TA+AA	GG	TT	0.001	3.03 (1.60～5.73)
TA+AA	GG	TC+CC	0.123	6.06 (0.615～59.7)
TA+AA	GA+AA	TT	-	-
TA+AA	GA+AA	TC+CC	0.025	2.45 (1.12～5.37)

FTO: fat mass and obesity-associated; MC4R: melanocortin 4 receptor; CI: confidence interval; OR: odds ratio; - means non-significant.

identified using GMDR is illustrated in Figure 1. The mutant genotypes of *FTO* rs9939609, MC4R rs12970134, and *MC4R* rs17782313 could be the high risk exposure factors for obesity (Figure 1).

Logistic regression analysis

Associations between obesity and the eight different combinations of genotypes compared with *FTO* rs9939609 TT, *MC4R* rs12970134 GG, and *MC4R* rs17782313 TT are shown in Table 4. Interactions among these three genes that made larger contributions to this model were *FTO* rs9939609 TA/AA, *MC4R* rs12970134 GA/AA, *MC4R* rs17782313 TC/CC. Compared with the wild homozygous genotype, the model of *FTO* rs9939609 TA/AA, *MC4R* rs12970134 GA/AA, *MC4R* rs17782313 TC/CC appears to confer the increased risk of obesity susceptibility (OR=2.45, 95% CI=1.12–5.37). The estimated risk of obesity was significantly higher in individuals with *FTO* rs9939609 TT, MC*4R* rs12970134 GA/AA, and *MC4R* rs17782313 TC/CC (OR=2.39, 95% CI=1.44–3.98).

DISCUSSION

The minor allele frequencies of *FTO* rs9939609 and rs9935401 and *MC4R* rs12970134 and 17782313 were 0.127, 0.123, 0.212, and 0.242, respectively, which were similar to those shown in HapMap (http://www.hapmap.org) Chinese data and were much lower than those shown in European populations in HapMap. These data suggested racial differences. Thus, studying the multiple loci interaction in the Chinese population was necessary.

The *FTO* is a widely validated obesity susceptibility gene determined through a GWAS, and Frayling et al first reported that the *FTO* rs9939609 SNP was highly correlated to the British obesity.[22,23] This association was repeated and verified in the European population.[6,7,24,25] However, results of studies about effects of *FTO* on obesity in the Asian population are currently inconsistent. Results from Hsiao and Yang revealed the correlation between *FTO* SNPs and obesity.[26,27] Li and Horikoshi failed to identify the relationship between *FTO* and obesity.[28,29] A meta-analysis of the *FTO* rs9939609 polymorphism and overweight/obesity in Chinese children indicated this association and that the risk of overweight/obesity with the 'A' allele is higher.[30] In our study, we confirmed the previous research result that the risk of being overweight was 1.996 times higher in TA/AA genotype carriers than in TT genotype carriers (95% CI=1.23–3.23).[30] For the rs9935401, several studies have observed that this loci was closely related to obesity but rarely reported in China.[10-12] Moreover, we confirmed that

the risk of being overweight was 1.836 times higher in GA/AA genotype carriers than in GG genotype carriers (95% CI=1.13–2.98).

Besides the *FTO*, *MC4R* is another gene that is related with obesity. *MC4R* is located on the long arm of chromosome 18 and was first found in a single-gene severe obese population.[31] GWASs have demonstrated that the polymorphism of the 'A' allele on the downstream of *MC4R* rs12970134 plays an important role in the occurrence of obesity.[15-17] Shijiazhuang demonstrated that the *MC4R* rs17782313 polymorphism was related to overweight and obesity, and the CC genotype is an independent risk factor for obesity.[32] A study on Caucasian school children showed that the *MC4R* rs12970134 allele polymorphism was significantly correlated to overweight and obesity, and students with the AA genotype easily developed obesity and were overweight.[33] In the present study, we confirmed the previous result of the effect of rs12970134; the risk of being overweight was 1.72 times higher in GA/AA genotype carriers than in GG genotype carriers (95% CI=1.13–2.62). GWASs also demonstrated that the *MC4R* rs17782313 variation is a risk factor for body weight gain, body fat accumulation, and obesity.[34] *MC4R* rs17782313 is related to dietary energy and energy-dense macronutrient intakes in Chilean and Iranian adults.[35,36] This study also found that the risk of being overweight was 1.67 times higher in TC/CC genotype carriers than in TT genotype carriers.

Because *FTO* and *MC4R* and their site single nucleotide mutations play an important role in obesity and overweight, we applied GMDR to screen out the two-factor model (*FTO* rs9939609 and *MC4R* rs12970134) and the three-factor model (*FTO* rs9939609, *MC4R* rs12970134, and *MC4R* rs17782313), respectively. Their interaction for overweight was statistically significant ($p<0.05$), indicating that the three genes may affect interactively. The logistic regression analysis was further used to evaluate the risk of the model on children and adolescents being overweight. The risk of being overweight in was 2.39 times higher in *FTO* rs9939609 (TA/AA) -*MC4R* rs12970134 (GA/AA) carriers than in those with the wild homozygous types. In addition, the risk of being overweight was 2.45 times higher in *FTO* rs9939609 (TA/AA) -*MC4R* rs12970134 (GA/AA) -*MC4R* rs17782313 (TC/CC) carriers than in those with the wild homozygous type. These results demonstrated that these three genotypes have some connection and that they are associated with the incidence of obesity and overweight.

As mentioned above, *FTO* and/or *MC4R* were associated with obesity and overweight in all subjects. With the increasing number of the risk alleles of *MC4R* or *FTO*, the risk of obesity increased. In addition, compared with the subjects not carrying risk alleles, the subjects carrying more than two risk alleles were 2.39 times more likely to be obese. The results of our study are in agreement with the results of a previous study.[37]

In conclusion, the allele of *FTO* rs9939609, *FTO* rs9935401, and *MC4R* rs12970134 may be the risk factor for overweight in Ningxia. The combination of *FTO* rs9939609, *MC4R* rs12970134, and *MC4R* rs17782313 gene alleles has some effects on obesity in childhood. The results of this study provide a theoretical basis for the prevention and control of obesity.

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AUTHOR DISCLOSURES

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