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 rs935401 and MC4R rs12970134 and rs17782313 interactions were analysed through generalized multifactor dimensionality reduction analysis, 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. 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. 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. 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. 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. Moreover, obesity is likely to be a result of superimposition of the effects of different genes. 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). Significant interaction exists between the FTO polymorphism, energy consumption, and physical activi-

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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 (m²). 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/m² vs 17.8±2.47 kg/m²), 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 cm)
Table 1. Descriptive characteristics of overweight/obese and normal weight subjects

<table>
<thead>
<tr>
<th>Variants</th>
<th>Overweight/Obesity (n=170)</th>
<th>Normal weight (n=200)</th>
<th>T-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>156±15.6</td>
<td>149±16.1</td>
<td>4.27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.5±16.6</td>
<td>41.4±12.5</td>
<td>13.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Biceps circumference (cm)</td>
<td>24.9±3.69</td>
<td>20.8±3.13</td>
<td>9.58</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>80.5±11.6</td>
<td>70.8±9.44</td>
<td>7.44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>73.4±9.68</td>
<td>63.1±8.15</td>
<td>9.24</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>87.7±10.9</td>
<td>77.8±10.4</td>
<td>7.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7±3.90</td>
<td>17.8±2.47</td>
<td>19.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>111±13.7</td>
<td>107±13.8</td>
<td>2.40</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72.4±8.17</td>
<td>70.1±9.50</td>
<td>1.95</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body fat content (%)</td>
<td>24.2±5.22</td>
<td>18.6±8.40</td>
<td>5.70</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Basal metabolism (kcal)</td>
<td>1404±247</td>
<td>1261±227</td>
<td>4.78</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

BMI: body mass index. BMI was calculated as body weight (kg)/height (m²). 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=1.51, 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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Hardy-Weinberg equilibrium</th>
<th>Minor allele frequency (%)</th>
<th>Univariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>p</td>
</tr>
<tr>
<td>FTO rs9939609</td>
<td>T &gt; A</td>
<td>2/51/117</td>
<td>0.166</td>
</tr>
<tr>
<td>FTO rs9935401</td>
<td>G &gt; A</td>
<td>2/48/120</td>
<td>0.242</td>
</tr>
<tr>
<td>MC4R rs12970134</td>
<td>G &gt; A</td>
<td>8/69/93</td>
<td>0.283</td>
</tr>
<tr>
<td>MC4R rs17782313</td>
<td>T &gt; C</td>
<td>8/77/85</td>
<td>0.069</td>
</tr>
</tbody>
</table>

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

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

1X1 represent FTO rs9939609, X2 represent FTO rs9935401, X3 represent MC4R rs12970134, and X4 represent MC4R rs17782313.
Yan, XH Gao, XJ Tao, QH Gao, YH Zhang and JJ Yang

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, MC4R 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.

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

![Image](81x570 to 514x780)

**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

<table>
<thead>
<tr>
<th>FTO rs9939609</th>
<th>MC4R rs12970134</th>
<th>MC4R rs17782313</th>
<th>p-value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>TT</td>
<td>TT</td>
<td>0.000</td>
<td>0.495</td>
</tr>
<tr>
<td>TT</td>
<td>TG</td>
<td>TC+CC</td>
<td>0.625</td>
<td>1.29 (0.47–3.52)</td>
</tr>
<tr>
<td>TT</td>
<td>GA+AA</td>
<td>TT</td>
<td>0.488</td>
<td>2.02 (0.276–14.8)</td>
</tr>
<tr>
<td>TT</td>
<td>GA+AA</td>
<td>TC+CC</td>
<td>0.001</td>
<td>2.39 (1.44–3.98)</td>
</tr>
<tr>
<td>TA+AA</td>
<td>GG</td>
<td>TT</td>
<td>0.001</td>
<td>3.03 (1.60–5.73)</td>
</tr>
<tr>
<td>TA+AA</td>
<td>GG</td>
<td>TC+CC</td>
<td>0.123</td>
<td>6.06 (0.615–59.7)</td>
</tr>
<tr>
<td>TA+AA</td>
<td>GA+AA</td>
<td>TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA+AA</td>
<td>GA+AA</td>
<td>TC+CC</td>
<td>0.025</td>
<td>2.45 (1.12–5.37)</td>
</tr>
</tbody>
</table>

FTO: fat mass and obesity-associated; MC4R: melanocortin 4 receptor; CI: confidence interval; OR: odds ratio; - means non-significant.
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-genome severe obese population.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{34} MC4R rs17782313 is related to dietary energy and energy-dense macronutrient intakes in Chilean and Iranian adults.\textsuperscript{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 was 2.39 times higher in FTO rs9939609 (TA/AA) - MC4R rs12970134 (GA/AA) carriers than in those with the wild homozygous type. 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.\textsuperscript{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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