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Relationship Between Fat Mass and Obesity-Associated (FTO) Gene Polymorphisms with Obesity and Metabolic Syndrome in Ethnic Mongolians

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Background: The distribution of fat mass and obesity-associated gene (FTO) genes rs9939609 and rs1421085 in obese and normal ethnic Mongolians was analyzed to investigate the association of FTO gene polymorphisms with obesity and metabolic syndrome in ethnic Mongolians.

Material/Methods: The genotypes of FTO genes rs9939609 and rs1421085 in 500 subjects were detected by allele-specific PCR (AS-PCR). General characteristics and clinical biochemical indicators were compared between the obesity group and the control group. The correlation between different genotypes and obesity metabolic index was also analyzed.

Results: Body mass, body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), SBP, DBP, FPG, triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) were higher, while HDL-C was lower in the obesity group compared with controls. The frequencies of TT genotype and T allele in the obesity group were higher than those in the control group. The frequencies of these 3 genotypes and allele frequencies of Rs1421085 were comparable between the 2 groups (P>0.05). The risk of obesity in Mongolian individuals carrying rs9939609 AT genotype was 1.312 times higher and the risk in those carrying AA genotype was 1.896 times higher than in individuals with TT genotype. The body weight, BMI, WC, HC, and WHR in individuals with rs9939609 AA and AT genotypes were significantly higher than in those with TT genotype.

Conclusions: The AT/AA genotype and allele A of rs9939609 are associated with an increased risk of obesity.

MeSH Keywords: Metabolic Syndrome X • Obesity • Polymorphism, Genetic

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Background

With improvement of economy and lifestyle, the incidence of obesity has been increasing year by year and has become a problem that seriously endangers human health, burdens people's lives, and limits social development. Over 1 billion adults in the world are overweight and nearly 300 million adults are diagnosed as obese [1]. Obesity can not only cause hypertension, diabetes, and other metabolic diseases, but also has a serious effect on psychology, resulting in low self-esteem, eccentricity, and other behavioral disorders [2].

Obesity is a disease caused by many factors, such as environment and genetics. Some studies found that FTO is involved in the pathogenesis of obesity [3–7]. The human FTO gene is located on chromosome 16 (16q12.2) and its mRNA is mainly expressed in the hypothalamus, which controls energy balance. Therefore, the FTO gene may affect the occurrence of obesity through neuro-modulation, food-related genes, and eating behaviors [8–10]. A number of studies demonstrated that there is a close correlation between FTO single-nucleotide polymorphism (SNP) and the occurrence of obesity [11–13]. However, the polymorphic sites are different in different countries, nationalities, and age groups [10]. The FTO gene rs9939609 and rs1421085 genotypes have been identified in numerous large-scale trials that were related to obesity, BMI, and diabetes [5,12,14,15]. Therefore, the present study selected these 2 polymorphic sites for investigation.

As an ethnic minority area, Hohhot, Inner Mongolia has a large ethnic Mongolian population, as well as many ethnic Han people, with different lifestyles and diets. A previous study demonstrated that the age-standardized prevalence of obesity, denoted by the international BMI cutoff values, in men and women between 2005 and 2013 increased from 10.8% to 17.6% and from 18.9% to 26.4%, respectively. Using Asian-specific BMI cutoff values for men and women, the age-standardized prevalence of obesity between 2005 and 2013 increased from 20.0% to 32.8% and 33.4% to 43.7%, respectively [16], which was significantly higher than the prevalence of obesity worldwide (13% of the world's adult population, including 11% of men and 15% of women were obese in 2016). However, the distribution of FTO alleles in ethnic Mongolians and its correlation with obesity and metabolic syndrome remains poorly understood. This study investigated the relationship of the FTO gene in Mongolians with obesity and related metabolic syndrome, aiming to more effectively manage Mongolian obesity.

Material and Methods

Subjects

According to the diagnostic criteria of the 2003 edition of "Guidelines for Prevention and Control of Overweight and Obese Adults in China", 300 Mongolian overweight and obese patients from January 2017 to July 2017 at the Medical Examination Center of the Affiliated Hospital of Inner Mongolia Medical University were selected, including 150 males and 150 females, with a mean age of 35.82±6.54 years old. Another 200 non-overweight or obese subjects were enrolled as the control group, including 100 males and females, with an average age of 36.07±6.23 years.

General information

Data on gender, age, medical history, family history, smoking, drinking, diet, and exercise of all the participants were collected. Blood pressure, height, weight, WC, and HC were measured to calculate BMI=body weight (Kg)/height (m^2); WHR=WC/HC. A total of 4 ml EDTA anticoagulation venous blood was collected from each subject. FPG was measured by glucose oxidase method. TG, TC, HDL-C, and LDL-C were detected by automatic biochemical analyzer (AU 600 Olympus, Japan).

DNA extraction

A total of 300 μL anticoagulant blood was used to extract DNA using the blood DNA extraction kit (Tiangen Biochemical Technology Co., Ltd., Beijing), to which we added 750 μL of cell lysate CLA and centrifuged the mixture at 12 000 rpm for 1 min. Then, we added 198.5 μL FGA and 1.5 μL Proteinase K. Next, we added 200 μL isopropanol and centrifuged the mixture at 12 000 rpm for 5 min. Then, we added 300 μL of 70% ethanol and centrifuged it at 12 000 rpm for 2 min. Finally, the dry DNA sediment was dissolved in 50 μL buffer TB. DNA concentration and purity were measured on a NanoDrop 2000 UV spectrophotometer (Thermo, USA) and the sample was stored at –20°C.

PCR amplification

PCR reaction with 2 primers was performed to distinguish different genotypes. The PCR reaction system contained 12.5 μL 2×KOD buffer, 4 μL dNTPs, 0.5 μL F, 0.5 μL DNA R, 2 μL DNA, and 5.5 μL ddH_2O. PCR amplification was performed under the condition of pre-denaturation at 94°C for 2 min, followed by 16 cycles of 94°C for 30 s, 60–52°C for 30 s each cycle (–0.5°C), and 72°C for 30 s; followed by 24 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and 72°C for 10 min. Primer 5.0 was used to design primers. Primer sequences were:
609F: 5'CTGTATCTTTTGGCAGATCAG;

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Table 1. FTO gene rs9939609 and rs1421085 genotype and PCR product.

rs9939609	609F and 609tR	609F and 609aR	rs1421085	085F and 085tR	085F and 085cR
T/T genotype	+	–	T/T genotype	+	–
A/T genotype	+	+	C/T genotype	+	+
A/A genotype	–	+	C/C genotype	–	+

Table 2. General information and biochemical indicators comparison.

	Obesity group	Control	*t*	*P*
Age (year)	35.82±6.54	36.07±6.23	0.427	0.335
Height (cm)	166.73±7.08	165.94±7.65	1.183	0.119
Weight (kg)	74.68±11.92	59.83±7.27	17.288	<0.001
BMI (Kg/cm^2)	26.86±3.75	21.91±2.36	18.108	<0.001
WC (cm)	88.51±8.94	71.43±6.88	24.081	<0.001
HC (cm)	98.33±6.47	90.55±5.36	14.620	<0.001
WHR	0.90±0.06	0.78±0.07	19.863	<0.001
SBP (mmHg)	140.48±20.53	121.67±18.94	10.349	<0.001
DBP (mmHg)	89.72±14.06	77.61±10.85	10.842	<0.001
FPG (mmol/L)	5.18±1.74	4.39±0.91	6.622	<0.001
TG (mmol/L)	1.59±0.83	0.85±0.40	13.299	<0.001
TC (mmol/L)	4.71±1.10	4.32±1.14	3.828	<0.001
HDL-C (mmol/L)	1.22±0.51	1.54±0.58	3.729	<0.001
LDL-C (mmol/L)	2.70±0.84	2.09±0.62	9.331	<0.001

609aR: AGACTATCCAAGTGCATCACT3';
609tR: 5'AGACTATCCAAGTGCATCACA3';
085F: 5'ACTGTCTCTAAGCCCAACAAAC3';
085tR: 5'ATTCTCATCAGACACTTAATCAATA3';
085cR: 5'ATTCTCATCAGACACTTAATCAATG3'.

Agarose gel electrophoresis

A total of 5 μL of PCR product was mixed with 1 μL of 6×loading buffer, and then we added it into a 1.5% agarose gel and incubated it in a TBE electrophoresis buffer at 100 V for 40 min. The results were read by a gel electrophoresis imager and recorded radiographically. For different pairs of specific primers, different genotypes were analyzed according to the results of electrophoresis (Table 1).

Statistical analysis

All data analyses were performed using SPSS17.0 software. Measurement data are expressed as mean ± standard deviation and compared by *t* test. Enumeration data were depicted as the number of cases or rate and analyzed by χ^2-test. Risk assessment was performed using single-factor logistic regression analysis. P<0.05 was considered as statistical significance.

Results

Comparison of general information and biochemical indicators

There was no significant difference in age or height distribution between the 2 groups. Body mass, BMI, WC, HC, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), TG, TC, and LDL-C in the obese group were significantly higher than in the control group (Table 2).

FTO gene distribution frequency balance test

The frequency of FTO gene polymorphisms at 2 *loci* conformed to Hardy-Weinberg equilibrium (P > 0.05), indicating that the selected samples were representative (Table 3).

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Table 3. FTO gene distribution frequency balance test.

Locus	Allele	Genotype			Allele		χ^2	*P*
		AA (%)	AB (%)	BB (%)	A (%)	B (%)		
rs9939609	A/T	13 (2.6)	129 (25.8)	358 (71.6)	155 (15.5)	845 (84.5)	0.114	0.736
rs1421085	T/C	339 (67.8)	148 (29.6)	13 (2.6)	826 (82.6)	174 (17.4)	0.443	0.506

Table 4. rs9939609 allele and genotype frequency comparison.

	Locus	Genotype			χ^2	*P*	Allele		χ^2	*P*
		AA (%)	AT (%)	TT (%)			A (%)	T (%)		
Obesity group	rs9939609	9 (3.00)	89 (29.67)	202 (67.33)	6.715	0.035	107 (17.83)	493 (82.17)	6.235	0.013
Control		4 (2.00)	40 (20.00)	156 (78.00)			48 (12.00)	352 (88.00)		

Table 5. rs1421085 allele and genotype frequency comparison.

	Locus	Genotype			χ^2	*P*	Allele		χ^2	*P*
		AA (%)	AT (%)	TT (%)			A (%)	T (%)		
Obesity group	rs1421085	200 (66.67)	92 (30.67)	8 (2.67)	0.443	0.801	492 (82.00)	108 (18.00)	0.376	0.539
Control		139 (69.50)	56 (28.00)	5 (2.50)			334 (83.50)	66 (16.50)		

Table 6. Risk analysis of FTO gene rs9939609 polymorphism in Mongolian obesity.

Locus	Allele/ genotype	Obesity group	Control	χ^2	*P*	OR	95% CI
rs9939609	TT	202	156	6.715	0.035	1.000	
	AT	89	40			1.312	1.138~1.526
	AA	9	4			1.896	1.374~2.691
	T	493	352	6.235	0.013	1.000	
	A	107	48			1.904	1.257~2.354

Comparison of allele and genotype frequencies

Rs9939609 genotype frequency and allele frequency distribution were significantly different between the 2 groups (P<0.05). TT genotype and T allele frequency in the obese group was markedly higher than in the control group. However, there was no significant difference in rs1421085 genotype frequency and allele frequency distribution between the 2 groups (P>0.05) (Tables 4, 5).

Risk analysis of FTO gene rs9939609 polymorphism in Mongolian obesity

The Mongolian FTO gene rs9939609 was analyzed by one-way logistic regression analysis with TT genotype as a control. As shown in Table 6, the risk of obesity in Mongolian individuals carrying the rs9939609 AT genotype and AA genotype was 1.312 and 1.896 times higher, respectively than that of the TT genotype.

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Table 7. The relationship of rs9939609 polymorphism with obesity and related metabolic indicators.

	AA/AT	TT	*t*	*P*
Age (year)	166.18±6.84	165.96±6.33	0.275	0.392
Height (cm)	73.41±9.73	67.28±8.06	5.402	<0.001
Weight (kg)	26.58±3.04	24.43±2.62	6.319	<0.001
BMI (Kg/cm^2)	87.69±8.03	81.37±6.55	6.774	<0.001
WC (cm)	98.42±5.99	94.76±5.13	5.480	<0.001
HC (cm)	0.89±0.04	0.85±0.05	7.466	<0.001
WHR	133.27±21.53	131.26±20.14	0.792	0.214
SBP (mmHg)	84.61±11.38	83.52±10.19	0.989	0.162
DBP (mmHg)	4.58±1.71	4.47±1.23	0.569	0.285
FPG (mmol/L)	1.42±0.96	1.29±0.91	1.139	0.128
TG (mmol/L)	4.53±1.02	4.48±0.97	0.412	0.341
TC (mmol/L)	1.31±0.53	1.37±0.45	1.021	0.154
HDL-C (mmol/L)	2.19±0.88	2.32±0.71	1.275	0.102

The relationship of rs9939609 polymorphism with obesity and related metabolic indicators

The body weight, BMI, WC, HC, and WHR in individuals with rs9939609 AA and AT genotype were significantly higher than those with TT genotype. However, there was no significant difference in SBP, DBP, FPG, TG, TC, HDL-C, or LDL-C between genotype carriers and TT genotype carriers (P>0.05) (Table 7).

Discussion

Obesity is an important factor affecting physical and mental health. With increased incidence, obesity has become a global public health problem. Obesity is associated with several diseases, such as diabetes, hypertension, hyperlipidemia, and cardiovascular and cerebrovascular diseases [17,18]. In this study, the body weight, BMI, WC, HC, WHR, SBP, DBP, FPG, TG, TC, and LDL-C were higher, while HDL-C was lower in the obesity group compared with controls, confirming its role in the pathogenesis of obesity. Individual genetic analysis has been demonstrated to be more effective for use in screening susceptible populations and developing individualized intervention programs [19–21].

FTO is the first candidate gene associated with obesity and was discovered by genome-wide association studies (GWAS), providing the basis for scientific management and early intervention for people at risk of obesity. Several studies found that multiple SNPs of FTO are related to metabolic syndromes, such as obesity, BMI, and diabetes. However, the functional SNPs *loci* in different ethnic and age groups are not consistent. Blauw et al. enrolled 6990 participants 45–65 years old in the Netherlands and observed that rs1421085-CC carriers had a higher prevalence of obesity than TT carriers, with a higher BMI of 0.56 (0.15, 0.98) kg/m^2, WC of 1.25 (0.02, 2.49) cm, and overall weight of 1.21 (0.28, 2.14) kg [22]. Wu et al. selected 401 Chinese adolescents from 14 to 18 years old and analyzed multiple SNPs of the FTO gene. They found that BMI in the rs9939609 TA+AA, rs8050136 AC+AA, rs1558902 TA+AA, and rs3751812 GT+TT carriers was significantly higher than in WT TT genotype (rs9939609: P=0.038; rs1558902: P=0.038), CC genotype (rs8050136: P=0.024), and GG genotype (rs3751812: P=0.024) carriers [23]. However, these sites were independent of metabolic indicators, including blood glucose and lipid. Albuquerque et al. investigated Turkish children and found that rs9939609 and rs1421085 were closely related to weight, BMI, WC, and HC [24]. However, Mustafa Solak et al. showed no significant effects of rs1421085 and rs9939609 on Turkish obesity [14]. In addition to multiple large-scale studies demonstrating that rs1421085 and rs9939609 are associated with obesity, it was also confirmed that rs1421085 and rs9939609 can influence BMI through various factors, such as dietary behaviors, physical activity, food intake, and mental health [8,25]. Przeliorz-Pyszczek et al. suggested that rs1421085 can regulate the differential expression of IRX3 and IRX5 to affect the occurrence of obesity [26]. Therefore, the present study selected rs1421085 and rs9939609 to investigate their relationship with obesity in Mongolia.

In this study, we found that 3 genotype frequencies and allele frequencies distribution of rs9939609 in the obesity group were significantly different from in controls. The frequencies

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of TT genotype and T allele in the obesity group were higher than in the controls. However, the genotype frequencies of 3 genotypes and allele frequencies of Rs1421085 were comparable between the 2 groups, suggesting that rs9939609 may be related to the occurrence of obesity in ethnic Mongolians. Furthermore, we assessed the risk of rs99396093 genotype frequency and allele frequency on obesity. The risk of obesity in Mongolian individuals carrying the rs9939609 AT genotype and AA genotype was 1.312 and 1.896 times higher, respectively, than that of TT genotype. The obese risk of A allele was 1.904 times higher than that of T allele. In the UAE population, it was observed that rs9939609 was significantly associated with BMI (P=0.028), and A allele had a significant additive effect on BMI [15]. The effect of AA genotype on BMI can be reduced by physical activity (P=0.027). Body weight, BMI, WC, HC, and WHR of rs9939609 AA and AT genotype carriers were significantly higher than those of TT genotype carriers, which was consistent with previous reports [5]. Kim et al. found that rs9939609 polymorphism can affect dietary intake of fatty acids, of which the percentage of saturated fatty acids in total dietary energy was 12.6% higher in AA genotype than the TT genotype [9], and this may be one of the main causes of increasing risk of obesity in people with the AA genotype. In addition, many studies also confirmed that rs9939609 polymorphism is associated with physical activity and dietary intake and may influence the incidence of obesity through these behaviors [9,27]. However, the exact mechanism by which allele A is associated with the risk of obesity in Mongolians remains unclear and requires further investigations.

This study found that rs9939609 polymorphism was significantly associated with the occurrence of obesity in ethnic Mongolians, which may occur through regulation of energy metabolism. In addition, this study only selected a small part of the population in Hohhot, but not Mongolian people in other regions, and this is a certain bias in the sample selection. Therefore, large-cohort clinical studies are required to confirm this finding.

Conclusions

In this study, we demonstrated that the AT/AA genotype and the allele A of rs9939609 are related to the increased risk of obesity.

Conflict of interest

None.

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