Original Research Paper

International Physiology
Vol. 4 No. 2, July - December 2016
DOI: https://dx.doi.org/10.21088/ip.2347.1506.4216.1

Association of rs9939609 and rs1421085 with Obesity Risk in North Indian Population

Apurva Srivastava*,, Neena Srivastava*, Balraj Mittal**, Jai Prakash*, Pranjal Srivastava***, Nimisha Srivastava******

Abstract

Introduction: Obesity is progressively important health problem worldwide as well as in developing countries like India. Recent genetic studies have suggested that obesity associated FTO genetic variants are associated to obesity risk. *Aim:* To evaluate the association of FTO genetic variants towards obesity risk. *Subjects and Methods:* North Indian individuals categorized as non-obese (BMI<25 kg/m^2) and obese (BMI≥30 kg/m^2) were selected. FTO rs1421085, rs9939609 were genotyped by validated Taqman allelic discrimination. Their association with obesity was evaluated by means of single locus logistic regression by SPSS ver. 19. *Results:* In single locus analysis, significant association with obesity risk was revealed by FTO rs9939609 [CA (P=0.0001, OR=2.6(1.7-3.9); AA (P=0.0001, OR=3.0(1.7-5.3)], rs1421085 [TA (P=0.0001, OR=2.6(1.7-3.9); AA (P=0.0001, OR=2.8(1.6-5.2)]. *Conclusion:* This study to reveals that genetic variants of FTO are associated with obesity risk in North Indians.

Keywords: BMI; FTO; Obesity.

Introduction

The prevalence of obesity is increasing rapidly worldwide. The primary cause of the present outbreak is an unhealthy lifestyle, especially high calorie intake and poor physical activity. However, studies have established that the pathogenesis of obesity also includes a genetic component predisposing some individuals to gain more weight from a sedentary lifestyle (1–3). Fat mass and obesity-associated protein also known as alpha-ketoglutarate-dependent dioxygenase FTO is an enzyme that in humans is encoded by the FTO gene located on chromosome 16. Certain variants of the FTO gene are reported to be correlated with obesity risk in humans [1].

A study of 38,759 Europeans for variants of FTO identified an obesity risk allele [2]. Simultaneously, a study in 2,900 affected individuals and 5,100 controls of French descent, together with 500 trios (confirming an association independent of population stratification) found association of SNPs in the very same region of FTO (rs1421085) [3]. In addition, variants in the FTO gene were further confirmed to be associated with obesity in two very large genome wide association studies of body mass index (BMI) [4,5].

Owing to such promising results worldwide in context to FTO gene and its association with obesity risk we planned our study to look for association of two important genetic variants of FTO gene with obesity risk in our North Indian population.

Materials and Methods

Study Population and Ethics Statement

Individuals recruited in the study were of north Indian origin belonging to states of Delhi, Haryana, Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Punjab and Uttarakhand. Individuals other than North Indian origin were excluded. Informed written consent was obtained from each participant. The study was carried out after approval from local ethics committee of King George's Medical University, Lucknow, Uttar Pradesh, India. The study conforms to the code of ethics of the world medical association (64th WMA International Code of Medical

Author's Affiliations: *Department of Physiology, King George's Medical University, Chowk, Lucknow, Uttar Pradesh, India. **Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. ***Darbhanga Medical College and Hospital, Karpuri Chowk, Benta Laheriasarai, Darbhanga, Bihar, India,. ****Sikkim Manipal Institute of Medical Sciences (SMIMS), Upper Tadong, Tadong, Gangtok, Sikkim, India.

Corresponding Author: Neena Srivastava, Professor, Department of Physiology, King George's Medical University, Chowk, Lucknow 226003, Uttar Pradesh, India.
E-mail: neenasrivastavakgmc@gmail.com

Ethics General Assembly, Fortaleza, Brazil, October 2013).

The participants were recruited by organizing health awareness camps in the Lucknow city and its rural surroundings with the help of social service organizations. All study participants were subjected to a careful screening program including assessment of detailed personal and family history, physical examination, determination of anthropometric measurements and biochemical profiles.

Based on BMI, individuals were categorized as normal (BMI<25) and obese (BMI≥30). Overall 289 male subjects and 191 female subjects were enrolled in the study befitting the strict inclusion/exclusion criteria mentioned below.

Inclusion Criteria (Obese Subjects)

Subjects having BMI≥30 kg/m^2, age of 20-42 years at time of interview and place of birth North India.

Inclusion Criteria (Non-Obese Subjects)

Subjects having BMI from 18.5 to 24.99 kg/m^2, age of 20-42 years and Place of birth North India.

Exclusion Criteria

Subjects not fulfilling the above inclusion criteria for obese and non-obese subjects at time of interview and or with congenital disorders, mental disorders, endocrine disorders like Myxoedema, Cushing's syndrome and cardiovascular disease were excluded.

Measurements

Body weight in kilograms with weighing scale (Mfd. by Sunshine Instruments, Coimbatore and Product name- Omron) and height in centimeters by a stadiometer (Mfd. by Anand medical export, New Delhi and Product name- Stadio) was measured for individuals wearing light clothing. Waist circumference was measured midway between the iliac crest and the lower costal margin all along with hip circumference in centimeters with the help of waist tape (ATICO Medical Pvt. Ltd, Ambala and Product name- Atico) (Table 1). Blood samples were taken for lipid profile and genotyping. Serum lipid profile was estimated by commercial kit (ERBA diagnostics Mannheim GmbH, Germany). Genomic DNA from Blood was isolated using Qiagen DNA extraction kit (QIAamp® DNA Blood Kit)

Genotyping

Genotyping of the SNPs was carried out using the validated Taqman® allelic discrimination protocol (Applied Biosystems®).

Genotyping Quality Control

Genotype calling from real-time PCR data was performed using an algorithm called best cycle genotyping algorithm (BCGA). The quality of assignment of individual samples to clusters was determined on the basis of silhouette values [6] .

Statistical Analysis

Single Locus Logistic Regression Analysis

Genotype and allele distribution was compared between obese and non-obese subjects using *chi-square test*. The independent segregation of alleles was tested for the Hardy-Weinberg equilibrium (HWE), comparing the observed genotype frequencies with those expected (*chi-square test*). For case-control studies, differences in genotype distributions were calculated applying log additive logistic regression model adjusted for sex and age. A two-tailed p-value of less than 0.05 was considered a statistical significant result.

All statistical analyses were performed using SPSS software version 19.0 (SPSS, Chicago, IL, USA).

Table 1: Single Locus logistic regression Analysis of the investigated FTO SNPs

S. No.	Gene	SNP Id	Non-obese n(%) (n=240)	Obese n(%) (n=240)	P-value	OR (95%CI)
1	FTO	rs9939609				
		CC	159(66.3)	100(41.7)		
		CA	57(23.8)	94(39.2)	**0.0001**	**2.6(1.7-3.9)**
		AA	24(10)	46(19.2)	**0.0001**	**3.0(1.7-5.3)**
2	FTO	rs1421085				
		TT	162(67.5)	104(43.3)		
		TA	56(23.3)	94(49.2)	**0.0001**	**2.6(1.7-3.9)**
		AA	22(9.2)	42(17.5)	**0.0001**	**2.8(1.6-5.2)**

Results

Case-control study was performed in 240 obese and 240 non-obese healthy subjects. All the studied FTO polymorphisms followed Hardy Weinberg equilibrium in the control population.

Table 1 shows single locus logistic regression analysis of the investigated FTO SNPs with obesity risk. The analysis revealed the significant associations (P <0.05) of the 2 SNPs with obesity risk in Indian population.

Strong association was exposed in genetic variants of FTO rs9939609 [CA (P=0.0001, OR=2.6(1.7-3.9); AA (P=0.0001, OR=3.0(1.7-5.3)], rs1421085 [TA (P=0.0001, OR=2.6(1.7-3.9); AA (P=0.0001, OR=2.8(1.6-5.2)].

Discussion

In the present study, we report association of two important SNPs in FTO gene with obesity BMI≥30kg/m^2 in population of north India.

A cluster of single nucleotide polymorphism (SNPs) in the first intron of the FTO gene was found to correlate with an increase in body mass index (BMI) in both children and adults, regardless of gender in multiple cohorts spanning multiple ethnicities[7]. Recently a meta-analysis included 12 eligible studies consisting 5,000 cases and 9,853 controls revealed that FTO rs9939609 polymorphism was significantly associated with the increased risk of obesity in co-dominant model (AA vs. TT: OR = 1.91, 95% CI: 1.47-2.48, p < 0.01; AT vs. TT: OR = 1.18, 95% CI: 1.02-1.38, p = 0.03), dominant model (AA + AT vs. TT: OR = 1.47, 95% CI: 1.35-1.59, p < 0.01), recessive model (AA vs. AT + TT: OR = 1.79, 95% CI: 1.47-2.17, p < 0.01), and allelic model (A vs. T: OR = 1.39, 95% CI: 1.22-1.58, p < 0.01) which concluded that FTO rs9939609 polymorphism is associated with the increased risk of obesity among children and adolescents, especially the homozygous carriers [8]. Also, a study in Chinese Han children and adolescents found that the FTO rs9939609 variation is significantly associated with the risk of obesity and a meat-based dietary preference [9] which reveals that dietary preferences are also controlled by genes leading to obesity.

Many previous studies have also reported strong association between single nucleotide polymorphisms (SNPs) in the first intron of the fat mass and obesity-associated gene (FTO), on the chromosome 16q12.2 and risk to obesity, of which the rs9939609 is one of the most broadly studied, explaining on 1% of BMI heritability. The risk allele A of this genetic variant is responsible for 1.5 kg increase of body weight in human beings [2]. Significant increase in BMI was observed with rising numbers of A-alleles of rs9939609 in the COBRA study [0.52 kg/m^2 (95% CI 0.15–0.89); P = 0.006] and the UKADS/DGP study [0.42 kg/m^2 (95% CI 0.16–0.68); P = 0.002] along with combined meta-analysis of these two studies [0.45 kg/m^2 (95% CI 0.24–0.67); P = 3.0×10^{-5}] [10].

In a meta-analysis Significant associations were detected between obesity risk and the polymorphisms: rs9939609 (OR: 1.31, 95% CI: 1.26 to 1.36) and rs1421085 (OR: 1.43, 95% CI: 1.33 to 1.53) [11]. Evidences have also proved that the previously reported common polymorphisms rs9939609 and rs1421085 in FTO gene increase the risk of obesity in the Portuguese children [12]. In an association analysis between the FTO gene variant rs1421085 and risk of childhood obesity CC and CT/CC genotypes were associated with 59% and 71% increased risks of childhood obesity (adjusted OR = 1.59, 95%CI = 1.00-2.53 for CC; adjusted OR = 1.71, 95%CI = 1.10-2.65 for CT/CC) suggesting that variant rs1421085 in the FTO gene contributed to childhood obesity in Chinese children [13].

Recently, studies have reported that association of SNPs in FTO with obesity might be due to linkage between FTO intronic variations and some other genes. Ragvina et al. [14] reported that the obesity-associated SNPs rs8050136, rs1421085, rs9939609, and rs17817449 in FTO regulate IRX3 gene which is located several mega base away from FTO. Similarly, Smemo et al. [15] have also reported that variants within FTO interact through the promoters of IRX3 gene regulating its expression and determining obesity. In one of our recent studies we have also reported that genetic variants rs9939609, rs1421085 of FTO and rs3751723 of IRX3 genes are in high linkage disequilibrium (LD) and are associated with obesity risk in North Indians [16].

The studies carried out in background of FTO rs9939609 and rs1421085 have strongly established the role of these particular genetic variant to obesity risk and correspondingly in our present study consistent results were replicated for these genetic variants leading to high obesity risk in North Indian Individuals. Therefore, based on the present study we suggest that both the variants rs9939609 and rs1421085 in the FTO gene can be used as strong biomarkers to predispose obesity risk.

Acknowledgements

The authors acknowledge all the participants of the study and Department of Biotechnology, New Delhi, India, SAN No 102/IFD/SAN/PR-2015/2010-2011 dated 09-12-2010. Sanction orders Serial No: 96 and Indian council of Medical Research, New Delhi, India. Project no. 3/1/2(18)/OBS/2013/NCD-II.

References

1. Loos RJ, Yeo GS (2014). "The bigger picture of FTO: the first GWAS-identified obesity gene". Nat Rev Endocrinol. 2014; 10(1): 51–61.
2. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007; 316: 889–894.
3. Dina C., Meyre D., Gallina S., Durand E., Korner A., Jacobson P. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat. Genet. 2007; 39 (6): 724-726.
4. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, SulemP, Helgadottir A. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet. 2009; 41: 18–24
5. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet. 2009; 41: 25–34
6. Peter J. Rousseeuw. "Silhouettes: a Graphical Aid to the Interpretation and Validation of Cluster Analysis". Computational and Applied Mathematics. 1987; 20: 53–65.
7. J.A. Jacobsson, H.B. Schiöth, R. Fredriksson The impact of intronic single nucleotide polymorphisms and ethnic diversity for studies on the obesity gene FTO Obes. Rev. 2012; 13: 1096–1109
8. Quan LL, Wang H, Tian Y, Mu X, Zhang Y, Tao K. Association of fat-mass and obesity-associated gene FTO rs9939609 polymorphism with the risk of obesity among children and adolescents: a meta-analysis. Eur Rev Med Pharmacol Sci. 2015 Feb; 19(4): 614-23.
9. Min Yang , Yuyang Xu , Li Liang , Junfen Fu , Feng Xiong , Geli Liu , Chunxiu Gong , Feihong Luo , Shaoke Chen , Chunxiao Xu , Dandan Zhang , Zhengli Li , Shuai Zhang. The Effects of Genetic Variation in FTO rs9939609 on Obesity and Dietary Preferences in Chinese Han Children and Adolescents. PLoS One. 2014 Aug 11; 9(8): e104574.
10. Rees SD, Islam M, Hydrie MZ, Chaudhary B, Bellary S, Hashmi S et al. An FTO variant is associated with Type 2 diabetes in South Asian populations after accounting for body mass index andwaist circumference. Diabet Med. 2011; 28(6): 673–680.
11. Peng S, Zhu Y, Xu F, Ren X, Li X, Lai M. FTO gene polymorphisms and obesity risk: a meta-analysis. BMC Med. 2011 Jun 8; 9: 71.
12. Albuquerque D, Nóbrega C, Manco L. Association of FTO polymorphisms with obesity and obesity-related outcomes in Portuguese children. PLoS One. 2013; 8(1): e54370.
13. Wang L, Yu Q, Xiong Y, Liu L, Zhang X, Zhang Z, Wu J, Wang B. Variant rs1421085 in the FTO gene contribute childhood obesity in Chinese children aged 3-6 years. Obes Res Clin Pract. 2013; 7(1): e14-22.
14. Ragvina A, Moroc E, Fredmand D, Navratilovae P, Drivenese O, Engstromd PG et al. Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4 and IRX3. PNAS. 2010; 2: 775-780.
15. Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gomez-Marin C et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature. 2014; 507(7492): 371-5.
16. Apurva Srivastava, Balraj Mittal, Jai Prakash, Pranjal Srivastava, Nimisha Srivastava & Neena Srivastava. Association of FTO and IRX3 genetic variants to obesity risk in North India. Ann Hum Biol. 2015. Early Online: 1–6.