Original Article

Association of Fat Mass and Obesity-associated Gene Variant with Lifestyle Factors and Body Fat in Indian Children

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Abstract

Context: Common intronic variants of the fat mass and obesity-associated (FTO) gene have been associated with obesity-related traits in humans. **Aims:** (1) The aim of this study is to study the distribution of FTO gene variants across different body mass index (BMI) categories and (2) to explore the association between FTO gene variants and lifestyle factors in obese and normal weight Indian children. **Subjects and Methods:** Fifty-six children (26 boys, mean age 10.3 ± 2.2 years) were studied. Height, weight, and waist and hip circumference were measured. Physical activity (questionnaire) and food intake (food frequency questionnaire) were assessed. Body fat percentage (%BF) was measured by dual-energy X-ray absorptiometry. FTO allelic variants at rs9939609 site were detected by SYBR Green Amplification Refractory Mutation System real-time polymerase chain reaction using allele-specific primers. Generalized linear model was used to investigate the simultaneous influence of genetic and lifestyle factors on %BF. **Results:** Mean height, weight, and BMI of normal and obese children were 130.6 ± 7.1 versus 143.2 ± 15.6, 24.0 ± 5.2 versus 53.1 ± 15.8, and 13.9 ± 2.1 versus 25.3 ± 3.2, respectively. The frequency of AA allele was 57% among obese children and 35% in normal weight children. Children with the AA allele who were obese had least physical activity, whereas children with AT allele and obesity had the highest intake of calories when compared to children who had AT allele and were normal. %BF was positively associated with AA alleles and junk food intake and negatively with healthy food intake and moderate physical activity. **Conclusions:** Healthy lifestyle with high physical activity and diet low in calories and fat may help in modifying the risk imposed by FTO variants in children.

Keywords: Body mass index, children, diet, fat mass and obesity-associated, Indian, obesity, physical activity

Introduction

There has been an unmitigated rise in the prevalence of obesity in children, and it has been one of the most alarming public health issues facing the world today.[1] Among Indian children, various studies report the magnitude of overweight to be from 9% to 27.5% and that of obesity from 1% to 12.9%.[2-6] There is evidence of a demographic, epidemiological, and nutrition transition in India that is fuelling the epidemic of chronic diseases and obesity.[7]

Obesity is believed to be caused by a complex interplay of genes and environment. Common intronic variants of the fat mass and obesity-associated (FTO) gene have been found to be robustly associated with obesity-related traits in humans.[8] Among all the genome-wide association studies to identify obesity-associated loci, FTO variants have been reported to have the strongest influence on obesity and contribute maximally to the variance in body mass index (BMI).[9]

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The genetic predisposition for obesity in most individuals has a polygenetic basis. A polygenic variant by itself has a small effect on the phenotype; only in combination with other predisposing factors does a phenotypic effect arise. The extent to which lifestyle factors may modify this genetic risk is, however, unclear.[10] Some studies have suggested that the obesogenic effects of FTO may be accentuated by lower physical activity or blunted by higher physical activity, whereas other studies found no interaction between FTO and activity. Although there are few Indian studies which describe the prevalence of FTO gene, associations with environmental factors have been scarcely studied.

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Hence, our objectives were to study (1) the distribution of the FTO gene variants across different BMI categories and (2) the association of FTO gene variants and lifestyle factors in obese and normal weight Indian children.

Subjects and Methods

In a cross-sectional study, obese and normal weight children and adolescents were recruited from private clinics and health checks at the Jehangir Hospital in Pune city, India, on a voluntary basis. All children and parents were the inhabitants of Pune city and surrounding suburbs. A written informed consent was obtained from parents and assent from children prior to the commencement of the study. The research protocol was approved by the Ethics Committee of Hirabai Cowasji Jehangir Medical Research Institute, Pune, India. A pediatrician performed a clinical examination and children with endocrine disorders other than nutritional obesity were excluded from the study. Fifty-six children with the mean age 10.3 ± 2.2 years (26 boys) meeting the selection criteria were enrolled in the study.

Anthropometry

Standing height was measured to the nearest millimeter using a portable stadiometer (Leicester Height Meter, Child Growth Foundation, UK), and weight was measured using an electronic scale to the nearest 100 g. BMI was calculated by the formula weight/height in meter square. Height, weight, and BMI were converted to Z scores using contemporary Indian references.[11]

Waist circumference was measured in standing position using a stretch resistant tape; the tape also provided a constant 100 g of tension through the use of a special indicator buckle. The tape was applied horizontally just above the uppermost lateral border of the right ilium. The measurement was made at the end of normal expiration and was recorded to the nearest 0.1 cm. Hip circumference was measured at the widest portion of the buttocks.[12]

Diet and physical activity

A 24 h recall and validated food frequency questionnaire were used to record diet for all children.[13] Physical activity was assessed by QAPACE school children questionnaire[14] which was adapted for Indian children and adolescents' lifestyle.

Body composition

Body composition for all children was assessed using Lunar DPX-PRO total body pencil beam densitometer (GE Healthcare, Wisconsin, USA) using a medium mode scan (software encore 2005 version 9.30.044). The precision of repeat measurements in children with DPX Pro is reported to be 2.0% for fat mass (GE, Lunar).[15] %BF, android fat, gynoid fat, and total lean mass were calculated for all children.

Genetics (allelic discrimination real-time polymerase chain reaction)

FTO allelic variants were detected by SYBR Green Amplification Refractory Mutation System real-time polymerase chain reaction (RT-PCR) using allele-specific primers. Primers for the rs9939609 site polymorphisms (T/A) were designed using Primer Express v2 (Applied Biosystems, USA). The common forward primer has a sequence "AATTATTATTCTAGGTTCCTTGCGACTGCTG," while the allele-specific reverse primers are "TTAGAGTAACAGAGACTATCCAAGTGCATCATA" for the "T" allele and "TTAGAGTAACAGAGACTATCC AAGTGCATCATT" for the "A" allele. An additional mismatch (C > T) was introduced at the second last base at the 3' end of the allele-specific primers to increase primer stringency. Two parallel reactions were carried out with each of the allele-specific primers with the final volume of 10 μl each containing 200 nM of forward and reverse primers (Custom Synthesis Integrated DNA Technologies, IA, USA), 0.1 μM dNTPs and 0.1 μM dUTP to minimize carryover contamination (both Epicenter, WI, USA), 2.5 mM $MgCl_2$ (Invitrogen, CA, USA), 0.5U Taq polymerase, 1X PCR buffer (both Bangalore GeNei, India), and Uracil-N-glycosylase (UNG) 0.1 U (Invitrogen, CA, USA). RT-PCR was carried out in a Rotor-Gene 3000 (Corbett/Qiagen, Australia). PCR cycling conditions were as follows: UNG incubation at 37°C for 10 min, UNG inactivation and target denaturation: 94°C for 10 min followed by 38 cycles of denaturation at 95°C for 10 s, annealing at 65°C for 20 s with fluorescence acquisition on the FAM/SYBR channel, and extension at 72°C for 10 s. Melt curve data were collected by continuously monitoring fluorescence over a temperature ramp of 60°C–95°C at a rate of 0.5°C/ramp step. The melting peak was determined using a second derivative plot of the temperature fluorescence data. A melting peak of 72°C was noted as a specific signature for the amplicons and was used to determine specific amplification in each reaction. Samples that amplified only with the "T" primer were counted as "TT homozygotes," those that amplified with the "A" primer were counted as "AA homozygotes," while those that amplified equally with the "T" and "A" primers were counted as "AT heterozygotes."

Statistical analysis

All statistical analyses were carried out using the SPSS for Windows software program, version 12 (SPSS Inc, SPSS, Chicago, IL, USA). All outcome variables were tested for normality before performing any statistical analysis. Differences in mean were tested using Student's *t*-test. A $P < 0.05$ was considered significant. One-way ANOVA was used to assess the difference between the categories made according to the gene variants and BMI combined. The factors which influenced FTO allele and BMI categories in combination were analyzed using multinomial regression. Generalized linear model was used to study the association of FTO gene variant and lifestyle factors with %BF.

Results

Children were divided into obese and normal weight categories according to BMI cutoffs for their respective age-gender

groups.[16] The mean age of normal weight children ($n = 18$) was 9.7 ± 1.4 year and of obese children ($n = 27$) was 10.8 ± 2.6 year. There was a significant difference in the height, weight, and BMI between the two groups (130.6 ± 7.1 vs. 143.2 ± 15.6 cm, 24.0 ± 5.2 vs. 53.1 ± 15.8 kg and 13.9 ± 2.1 vs. 25.3 ± 3.2). In addition, waist and the hip circumferences measured were significantly higher in obese children ($P < 0.01$) [Table 1]. The %BF was more than double in obese children as compared to normal weight children (17.2 ± 7.5% vs. 41.4 ± 8.2). There was a significant difference in the physical activity performed by children in both the categories. Obese children were engaged in moderate physical activity only for 37 ± 30 min per day, whereas normal weight children played for 72 ± 37 min per day ($P < 0.05$). The diet recorded in both the groups showed that there were no significant differences in the total calories consumed by both the groups, but the diet of obese children was rich in fats and proteins ($P < 0.05$). The mean calorie intake of normal children was 1201 ± 432 Kcal, whereas that of obese children was 1540 ± 550 Kcal. The mean fat intake in diet of normal children was 37 ± 15 g and in obese children's diet was 60 ± 25 ($P < 0.05$) [Table 2]. The distribution of the FTO gene allele has been described in Figure 1. The risk homozygous AA allele was found to be 62% in the obese group, while 38% in the normal children. While TT allele was distributed as 66% in obese children and 34% in normal, and the heterozygous AT was 56% in obese children and 44% in normal children.

Further, when children with the A allele (AA and AT) were divided into four subgroups-AA and obese, AA and normal, AT and obese, and AT and normal, it was observed that the group AA and obese were the least active as measured by moderate activity per day and children who had the AT allele and were obese consumed significantly higher calories as compared to children with AT allele and normal weight ($P < 0.05$) [Table 3].

The association of total %BF with A alleles and diet was performed using generalized linear model. The analysis showed a significant positive association of total %BF with A alleles ($\beta = 0.144$, $P < 0.05$) and high-calorie foods ($\beta = 0.004$, $P < 0.05$) and negative association with healthy food intake ($\beta = 2212–0.012$, $P < 0.05$) and physical activity ($\beta = -0.059$, $P < 0.05$).

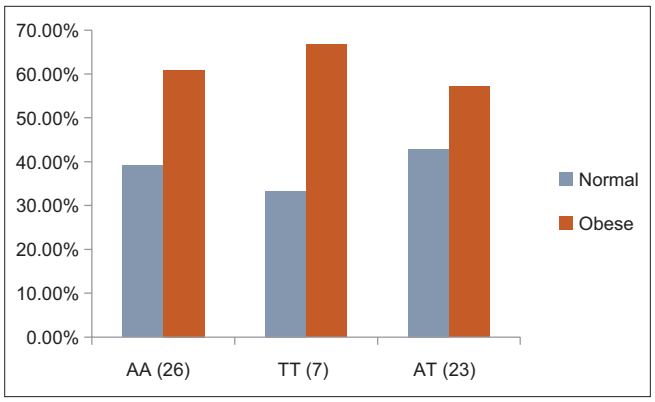

Figure 1: Distribution of fat mass and obesity-associated alleles among children

Discussion

Our results indicate that there was a strong association between the A allele and lifestyle factors. The impact of the FTO gene seems to be altered strongly by diet and physical activity. The diet of obese children in our study was significantly higher in fat and proteins. Further, physical activity was significantly lower among them. Our data are in keeping with the findings of Sonestedt *et al*.[17] and Kilpeläinen *et al*.[18] who have also suggested that high-fat diets and low physical activity levels may accentuate the susceptibility to obesity by the FTO variant, and lifestyle modifications may be an effective way of controlling body weight in individuals with a genetic predisposition toward obesity, supporting the fact that genetic influences are modifiable. The distribution of the risk allele (AA) was 35% among normal weight children and 57% among obese children. When children with only the A allele (AT and AA) were categorized according to their BMI, it was again noted that obese children consumed significantly higher calories and had lower physical activity. This indicates that although the normal weight children were also at risk of obesity, following a healthier lifestyle had helped in reducing the risk of obesity in them.

There is a continuous debate on whether lifestyle factors can modify the genetic risk of obesity. Some studies have shown the impact of lifestyle factors modifying the risk,[8,18-22] whereas other studies have not seen the same effect.[10,23] A diet low in calories combined with high physical activity has shown to

Table 1: Anthropometry of the study population

	Normal (18)	Obese (27)
Age (year)	9.7±1.4	10.8±2.6
Height (cm)	130.6±7.1	143.2±15.6*
HAZ	−0.8±1.3	0.2±1.1*
Weight (kg)	24.0±5.2	53.1±15.8*
WAZ	−1.4±1.2	1.5±0.6*
BMI (kg/m^2)	13.9±2.1	25.3±3.2*
BAZ	−1.3±1.1	1.7±0.5*
Waist (cm)	56.5±7.0	81.9±9.5*
Hip (cm)	66.2±6.5	90.8±10.8*
Total body fat percentage	17.2±7.5	41.4±8.2*

*$P<0.05$, mean of obese children significantly higher than normal children. HAZ: Height-for-age *Z*-score, WAZ: Weight-for-age *Z*-score, BAZ: BMI-for-age *Z*-score

Table 2: Diet and physical activity

	Normal (18)	Obese (27)
Calories (kcal)	1201±432	1540±550
Protein (g)	25±10	40±17*
Fat (g)	37±15	60±25*
Moderate activity (min/day)	72±37	37±30*

*$P<0.05$, mean significantly different between obese and normal children

Table 3: Classification of children according to fat mass and obesity allele and body mass index categories combined

	AA + obese (14)	AA + normal (9)	AT + obese (13)	AT + normal (9)
Calories (kcal)	1395±603	1179±356	1704±515*	1155±523
Protein (g)	35±16	25±11	44±18	24±12
Fat (g)	54±27	36±14	64±26	36±19
Physical activity (min/day)	35±20*	67±24	54±53	73±49

*P<0.05, mean significantly different as compared to AT + normal group

be helpful in modifying the genetic risk in several studies.[17,18] Our study is in line with these studies where a significant impact of physical activity and diet on modifying the risk has been observed.

Reports indicate that there is an increased risk of obesity in a person with the risk allele who has a lower physical activity and higher intake of calories.[13] A similar trend was seen among our study population as well. These findings are highly suggestive of a role of lifestyle in modifying the association between FTO variants and obesity. Despite the fact that lifestyle has undergone a considerable change in India, Indian studies evaluating the effect of lifestyle factors on the FTO gene variants are very scarce.

When groups were made according to BMI and the gene variants, it was found that children who had the risk allele AA and were obese had the least physical activity and those children who had AT allele and were obese consumed significantly higher calories, further reinforcing the fact that the lifestyle factors also had a role to play. The other two groups also carried the risk allele A but had a healthier lifestyle. Similar results have also been reported by others[8] who also observed that with lifestyle modifications, the risk imposed by a gene can be modified.

To further study the impact of lifestyle factors, we studied the association of total %BF with the A allele. There was a positive association seen between both. The total %BF was also negatively associated with the physical activity and positively associated with junk food intake.

The distribution of the gene variants shows that the protective allele TT is also seen largely among the obese children. This could be due to the small sample size and more number of obese children in the study. A bigger sample size is needed to observe the effect of the variation of the alleles. Studies conducted in Indian children earlier have reported the risk of higher BMI in children with the AA allele, but our study is unique in that it looks at the association of lifestyle factors with the gene which has not been done before. In view of the rising epidemic of obesity and the nutrition transition in India, studying the factors which can modify the risk imposed by the gene is important so that strategies to combat obesity can be devised. The sudden shift toward obesity does tell us that there are environmental factors involved rather than only genes. FTO gene variants and their relationship with obesity clearly require further well-designed, long-term studies with larger sample sizes with taking into account the possible impact of epigenetics; further other metabolic parameters also need to be measured to confirm the results of this study.

Conclusions

To summarize, our findings are highly suggestive for a role of healthy lifestyle in modifying the association between FTO variants and obesity in Indian children.

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Conflicts of interest

There are no conflicts of interest.

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