Obesity Research & Clinical Practice 13 (2019) 137–142

Contents lists available at ScienceDirect

Obesity Research & Clinical Practice

journal homepage: www.elsevier.com/locate/orcp

Original Article

The role of the genetic variants *IRX3* rs3751723 and *FTO* rs9939609 in the obesity phenotypes of children and adolescents

Pâmela Ferreira Todendi[a], Andreia Rosane de Moura Valim[b], Elisa Klinger[b], Cézane Priscila Reuter[b], Susana Molina[c], J. Alfredo Martínez[d], Marilu Fiegenbaum[a,*]

[a] *Graduate Program in Pathology, Federal University of Health Sciences of Porto Alegre – UFCSPA, Porto Alegre, RS, Brazil*
[b] *Graduate Program in Health Promotion, University of Santa Cruz do Sul – UNISC, Santa Cruz do Sul, RS, Brazil*
[c] *IMDEA Food, Crta. de Canto Blanco no8, Madrid, Spain*
[d] *Department of Nutrition and Food Sciences, Physiology and Toxicology, University of Navarra, Irunlarrea 1, Pamplona, Navarra, CIBERobn and IMDEA Food, Madrid, Spain*

ARTICLE INFO

Article history:
Received 18 July 2018
Received in revised form
28 November 2018
Accepted 14 January 2019

Keywords:
Adiposity
Children
Adolescents
IRX3
FTO

ABSTRACT

Objective: We investigated the association of *IRX3* SNP rs3751723 with anthropometric characteristics related to adiposity and potential relationships with *FTO* SNP rs9939609 in a population of Brazilian children and adolescents.

Methods: A total of 871 children and adolescents between 7 and 17 years of age were recruited. Adiposity measurements and biochemical parameters were assessed. The variants were genotyped by real-time PCR. Analysis of multiple linear regression, multiple logistic regression, and generalised multifactor dimensionality reduction (GMDR) adjusted for sex, age and ethnicity were applied to test the polymorphisms association with obesity-related phenotypes and the interaction between them.

Results: The analyses showed that *IRX3* was associated with obesity and fat percentage (BF%). An association of *FTO* rs9939609 with body mass index (BMI) Z-Score and with waist circumference (WC) was detected. The odds ratios (OR) showed that *IRX3* rs3751723 was associated with risk of obesity in additive model ($p = 0.017$), recessive model ($p = 0.016$) and with high BF% in all models. *FTO* rs9939609 was associated with risk of obesity in additive model ($p = 0.031$), recessive ($p = 0.033$) and with altered WC in all models. GMDR-based predictive models for the risk of obesity, altered WC and high BF% adjusted by age, ethnicity and sex suggested no interaction of the two loci.

Conclusions: The genetic variants rs3751723 and rs9939609 have an influence on the characteristics of adiposity; however, the effects of *IRX3* and *FTO* investigated polymorphisms are independent in relation to adiposity parameters.

Introduction

Obesity is one of the greatest health problems in the world [1] It is linked to increases in the risk of hypertension, cardiovascular diseases, type 2 diabetes, and other diseases [2,3]. Furthermore, obesity is complex phenotype [4] with heritability estimates between 40 and 70%. Several genes that have expression in the hypothalamus and that play roles in regulating appetite, as well as diverse energy metabolic pathways, are known to be involved [5]. Genome-wide association studies (GWAS) showed reproducible associations of fat mass and obesity (*FTO*) intronic gene variants with a higher risk of obesity [6,7]. Although the molecular mechanisms that link *FTO* noncoding variants with obesity were not immediately obvious, subsequent studies in mice showed that FTO expression levels influence body composition phenotypes [8,9].

A recent study showed that noncoding sequences within the *FTO* gene associated with obesity are functionally connected at a 500 kb downstream distance with the homeobox 3 Iroquois (*IRX3*) gene [10]. *IRX3* gene is located on 16q12.2 and has four exons [11], is expressed in the neural tube, lateral mesoderm and early embry-

Abbreviations: BMI, body mass index; BF%, body fat percentage; FTO, fat mass and obesity; GMDR, generalised multifactor dimensionality reduction; GWAS, genome-wide association studies; IRX3, iroquois homeobox 3; SNP, single nucleotide polymorphisms; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHO, World Health Organisation.

* Corresponding author at: Department of Basic Health Sciences, Federal University of Health Sciences of Porto Alegre, Rua Sarmento Leite, 245/403, CEP 90050-170, Porto Alegre, RS, Brazil.

E-mail address: mariluf@ufcspa.edu.br (M. Fiegenbaum).

https://doi.org/10.1016/j.orcp.2019.01.005
1871-403X/

onic tissues [12]. However, its expression has also been functionally implicated in long-distance susceptibility loci associated with obesity [13]. Recently, it was demonstrated that *FTO* SNPs associated with obesity (especially rs8050136, rs1421085, rs9939609 and rs17817449) are within a region that regulates the *IRX3* gene [14]. A subsequent study with the variant rs3751723 which is located in the 5'UTR region of the *IRX3* gene, showed a strong linkage disequilibrium between *FTO*rs17817449-*IRX3*rs3751723, and also, a high-order gene-gene interaction for obesity (*FTO*rs9939609, *FTO*rs17817449 and *IRX3*rs3751723) [15].

In Brazil, the prevalence of overweight and obesity among adults is 52.5% [16]. This scenario is not different among children and adolescents, reaching 41% prevalence [17]. Brazil is a high admixture country, with Europeans, Africans and Native Americans [18] representing the main ethnic contributions. The population of southern Brazil, where this study occurs, is composed mainly of European descent, with a contribution of 8 to 13% of Africans [19]. The effect of the *FTO* gene on obesity in children and adolescents was shown in Brazilian populations [20,21], but none investigated the effect of *IRX3* and *FTO* gene variants. Considering that *FTO* SNPs effects on obesity may be linked to genetic variation of *IRX3* gene, our study aimed to analyse the association of *IRX3* SNP rs3751723 with anthropometric characteristics related to adiposity and its relation with the *FTO* rs9939609 in a population of Brazilian children and adolescents.

Materials and methods

Study design and subjects

Participants were recruited between March 2014 and December 2015. The participants were 871 children and adolescents between 7 and 17 years of age attending public and private schools in Santa Cruz do Sul, Rio Grande do Sul, Brazil and members of the Phase III School Health Study [22]. All students were invited to participate in the survey, and their parents or guardians signed a free informed consent form. The study included apparently healthy subjects, who did not have restrictions for blood collections. The study protocol was approved by the Research Ethics Committee of the University of Santa Cruz do Sul (UNISC) under number 714 216/14 and by the Federal University of Health Sciences of Porto Alegre (UFCSPA) under number 995 205/15. This cross-sectional study has a sample size representative of the total number of students enrolled in the 6th Regional Education Coordination of Rio Grande do Sul and the Municipal Department of Education of Santa Cruz do Sul. For the sample size calculation, the Nea Research Division [23] formula was applied: $s = X^2\ NP(1-P)/d^2\ (N-1) + X^2 P(1-P)$, was used. Where s is the required sample size, X^2 is the value of chi-square for one degree of freedom, N is the population size, P is the population proportion, and d is the degree of accuracy expressed as a proportion. The calculation suggested that, for the 20.380 students enrolled, the sample should consist of 392 students, with an error of 5%.

The population from Southern Brazil is ethnic mixed [24]; thus, the ethnicity determination was made according to Parra et al. [25] based on an evaluation of the following phenotypic characteristics: skin color in the medial part of the arm; color and texture of hair; and the shape of the nose and lips. In this framework, 75% of the subjects were determined to be European descendants.

Anthropometric and biochemical measurements

Birth weight was recruited nominally through a questionnaire self-referred by parents or guardians. The height (coupled stadiometer) and body weight were measured using a Welmy scale (Welmy: 15416, Santa Bárbara D'Oeste-SP, Brazil). Using the formula BMI = weight/height^2 (kg/m^2), the BMI of each participant was calculated. The body mass index (BMI) Z-score were analysed according to the World Health Organisation criteria based on sex and age, was categorised as ≤+1SD, normal; >+1SD, overweight; and >+2SD obese [26].

Waist circumference (WC) was measured using the parameters established by Taylor et al. [27], classified as the normal circumference (percentile ≤80) and the altered (percentile >80). Body fat percentage (BF%) was measured using a Lange® (Beta Technology Incorporated, Cambridge, USA) compass to measure triceps and subscapular cutaneous skin folds. Then, the equation of Slaughter et al. [28] was applied (considering sex, age and puberty) and the values were classified according to the criteria established by Lohman [29], in two classifications: (1) normal (boys <20%, girls <25%) and (2) high (boys ≥20%, girls ≥25%). The technicians responsible for the anthropometric measurements were previously trained by physical education professionals and remained the same throughout the collection.

Blood was collected by venipuncture after 12 h of fasting. Measurements of total cholesterol (TC), HDL-C cholesterol, triglycerides (TG) and glucose were performed using commercial kits from Kovalent (Kovalent do Brazil Ltda, São Gonçalo, Rio de Janeiro). The Friedewald equation [30] was used for the calculation of LDL-C cholesterol. Total cholesterol, LDL-C and TG were classified as normal and high (TC >199.9 md/dL, LDL-C >129.9 md/dL, TG >99.9 md/dL ≤9 years and >129.9 md/dL ≥10 years), while HDL-C was classified as normal and low (<39.9 md/dL) [31]. Glucose was considered normal at ≤99 mg/dL and as prediabetes at levels of 99.9 mg/dL to 125.9 mg/dL [32].

Genotyping

The salting-out method was used to extract DNA [33] from 500 μl of each blood sample. Subsequently, it was quantified in the NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, USA). Taqman™ allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) for genotyping the *FTO* polymorphisms rs9939609 and *IRX3* rs3751723 were performed according to the manufacturer's instructions. Genotype calling was performed with the Taqman Genotyper Software v1.3 (Applied Biosystems™). TaqMan™ assays C__27476879_10 (rs3751723) and C__30090620_10 (rs9939609) and Master Mix PCR Universal were purchased from Applied Biosystems (Foster City, CA, USA).

Statistical analyses

Continuous variables were expressed as the mean (±standard deviation). Allele frequencies were estimated by gene counting. The allelic and genotype distribution between groups and Hardy-Weinberg equilibrium were tested using the chi-square test. Linkage disequilibrium (D′ and r^2) between polymorphisms was calculated using Haploview 4.2 software [34]. Multiple linear regression adjusted for sex, ethnicity and age was used to evaluate the risk of *IRX3* and *FTO* genotypes on adiposity (BMI Z-Score, WC and BF%). Multiple logistic regression was carried out to estimate the *FTO* and *IRX3* genotypes risk for obesity-related phenotypes (obesity, altered WC and high BF%). Additive, dominant and recessive models were tested. Statistical analyses were performed using SPSS 23.0 for Windows (SPSS, Chicago, USA). To examine potential *FTO-IRX3* interaction, we applied the generalised multifactor dimensionality reduction (GMDR) approach and the model that maximised testing accuracy and cross-validation consistency (CVC) was choose as the best model (version 0.9, http://www.ssg.uab.edu/gmdr/). A p-value <0.05 was considered statistically significant.

Table 1
Qualitative and quantitative anthropometric and biochemical features of the participants (n = 871).

Variables	N (%)	*IRX3* rs3751723			P
		GG	GT	TT	
Sex					0.340
Female	481 (55.2)	196 (40.7)	233 (48.4)	52 (10.8)	
Male	390 (44.8)	171 (43.8)	170 (43.6)	49 (12.6)	
Age range					0.086
Child (7–9 years)	202 (23.2)	93 (46.0)	94 (46.5)	15 (7.4)	
Adolescent (10–17 years)	669 (76.8)	274 (41.0)	309 (46.2)	86 (12.9)	
Ethnicity					0.071
European ancestry	481 (71.4)	193 (40.1)	226 (47.0)	62 (12.9)	
Other ancestry	193 (28.6)	86 (44.6)	94 (48.7)	13 (6.7)	
Birth weight (kg)[a]					0.195
Normal	698 (90.2)	292 (41.8)	331 (47.4)	75 (10.7)	
Overweight	76 (9.8)	37 (48.7)	28 (36.8)	11 (14.5)	
BMI Z-score[b]					0.103
Normal weight	489 (56.2)	194 (39.7)	232 (47.4)	63 (12.9)	
Overweight	219 (25.2)	90 (41.1)	104 (47.5)	25 (11.4)	
Obese	162 (18.6)	83 (51.2)	66 (40.7)	13 (8.0)	
Waist circumference/WC (cm)[c]					0.532
Normal	687 (78.9)	283 (41.2)	322 (46.9)	82 (11.9)	
Altered	184 (21.1)	84 (45.7)	81 (44.0)	19 (10.3)	
Body fat percentage (BF%)[d]					**0.031**
Normal	561 (64.4)	222 (39.6)	264 (47.1)	75 (13.4)	
High	310 (35.6)	145 (46.8)	139 (44.8)	26 (8.4)	
Total Cholesterol (TC) (mg/dL)[e]					0.380
Normal	484 (56.1)	201 (41.5)	233 (48.1)	50 (10.3)	
High	379 (43.9)	164 (43.3)	167 (44.1)	48 (12.7)	
LDL Cholesterol (mg/dL)[f]					0.715
Normal	712 (82.5)	303 (42.6)	326 (45.8)	83 (11.7)	
High	151 (17.5)	62 (41.1)	74 (49.0)	15 (9.9)	
HDL Cholesterol (mg/dL)[g]					0.509
Normal	853 (98.8)	359 (42.1)	397(46.5)	97 (11.4)	
Low	10 (1.2)	6 (60.0)	3 (30.0)	1 (10.0)	
Triglycerides (TG) (mg/dL)[h]					0.133
Normal	615 (71.3)	250 (40.7)	288 (46.8)	77 (12.5)	
High	248 (28.7)	115 (46.4)	112 (45.2)	21 (8.5)	
Glucose (mg/dL)[i]					0.701
Normal	813 (94.2)	341 (41.9)	379 (46.6)	93 (11.4)	
High	50 (5.8)	24 (48.0)	21 (42.0)	5 (10.0)	

p-Value Chi-square test.
Significance of bold values P < 0,05.
[a] Birth weight: normal ≤3999 kg, Overweight ≥4000 kg.
[b] BMI Z-score: Low/Normal Weight <+1SD, Overweight >+1SD, Obese >+2SD.
[c] WC (according to sex and age): normal percentile ≤80 and altered percentile >80.
[d] Body fat percentage (BF%): normal (boys <20%, girls <25%) and high (boys ≥20%, girls ≥25%).
[e] high Total Cholesterol (TC) >199.9 mg/dL.
[f] high LDL Cholesterol >129.9 mg/dL.
[g] low HDL Cholesterol <39.9 mg/dL.
[h] high Triglycerides (TG) >99.9 mg/dL (<9 years) and >129.9 mg/dL (>10 years).
[i] high Glucose: ≥100 mg/dL.

Results

Socio-demographic, anthropometric and biochemical variables are described for all samples and according to *IRX3* genotypes in Table 1. The predominant sex was female (55%) and the majority were adolescents (76%). Overall, 43.8% of subjects were classified as overweight or obese using BMI Z-score cut-off values. Hypercholesterolemia (measured by total cholesterol levels) was found in 43.9% of the study sample. In this initial analysis, the *IRX3* TT genotype was more frequently observed among subjects with normal body fat percentage (BF%) ($p = 0.031$).

Genotypic distributions for *IRX3* rs3751723 and *FTO* rs9939609 were in agreement with Hardy-Weinberg equilibrium. No linkage disequilibrium was detected between *IRX3* rs3751723 and *FTO* rs9939609 SNPs ($D' = 0.07$, $r^2 = 0$).

A multiple linear regression model was constructed to identify the effect of the SNPs studied on the anthropometric characteristics of adiposity (Table 2). This analysis revealed that age was significantly associated with the three parameters of adiposity ($p < 0.05$) and that sex was associated with WC ($p = 0.020$) and BF% ($p < 0.001$). Ethnicity was not associated with obesity-related phenotypes (data not shown). The test also confirmed the association of *IRX3* rs3751723 with BF% (GG genotype $\beta = 2.341$, $p = 0.014$). An association of *FTO* rs9939609 with BMI Z-Score (TA genotype: $\beta = 0.217$, $p = 0.037$ and AA genotype: $\beta = 0.383$, $p = 0.014$) and with WC (AA genotype: $\beta = 3.233$, $p = 0.008$) was detected. No interaction was observed between *IRX3* rs3751723-*FTO* rs9939609 polymorphisms ($p = 0.576$ for interaction on BMI Z-Score; $p = 0.647$ for WC and $p = 0.599$ for BF%) (data not shown).

Table 3 shows the adjusted odds ratios (OR) for obesity, altered WC and high BF% according *FTO* and *IRX3* genotypes. *IRX3* rs3751723 was associated with risk of obesity in additive model (GG genotype, OR = 2.61, 95%CI = 1.18–5.76, $p = 0.017$), recessive model (GG × GT + TT genotypes, OR = 1.62, 95%CI = 1.09–2.39, $p = 0.016$) and with high BF% in all models (additive, dominant and recessive). *FTO* rs9939609 was associated with risk of obesity in additive model (AA genotype, OR = 1.89, 95%CI = 1.06–3.73, $p = 0.031$), recessive model (AA × AT + TT genotypes, OR = 1.77, 95%CI = 1.05–3.00, $p = 0.033$) and with altered waist circumference in all models (additive, dominant and recessive models). No inter-

Table 2
Multiple linear regression of BMI Z-Score, WC and BF% with sex, age and *IRX3* and *FTO* SNP genotypes.

Variable	β ± Standard error	P
BMI Z-Score[a]		
Constant	1.815 ± 0.297	<0.001
Female sex	−0.036 ± 0.097	0.709
Age (years)	−0.103 ± 0.017	<0.001
IRX3 rs3751723		
GT	0.214 ± 0.161	0.183
GG	0.296 ± 0.163	0.069
FTO rs9939609		
TA	0.217 ± 0.104	**0.037**
AA	0.383 ± 0.155	**0.014**
WC (cm)[b]		
Constant	50.996 ± 2.336	<0.001
Female sex	−1.793 ± 0.766	0.020
Age (years)	1.412 ± 0.136	<0.001
IRX3 rs3751723		
GT	1.440 ± 1.265	0.256
GG	1.692 ± 1.283	0.188
FTO rs9939609		
TA	1.294 ± 0.817	0.114
AA	3.233 ± 1.220	**0.008**
BF%[c]		
Constant	6.346 ± 1.737	<0.001
Female sex	5.953 ± 0.569	<0.001
Age (years)	0.280 ± 0.101	0.006
IRX3 rs3751723		
GT	1.242 ± 0.941	0.187
GG	2.341 ± 0.954	**0.014**
FTO rs9939609		
TA	0.536 ± 0.607	0.377
AA	1.361 ± 0.907	0.134

β: Coefficient of regression beta corresponding to an increase or a decrease in the analysed variables.
Reference genotypes: TT (*IRX3* rs3751723) and TT (*FTO* rs9939609).
Significance of bold values P < 0,05.
[a] Model r^2 = 0.067; *FTO* TA partial r^2 = 0.006; *FTO* AA partial r^2 = 0.009.
[b] Model r^2 = 0.151; *FTO* AA partial r^2 = 0.010.
[c] Model r^2 = 0.163; *IRX3* GG partial r^2 = 0.009.

action between *FTO* rs9939609- *IRX3* rs3751723 was observed (Table 3).

Additionally, we tested GMDR-based predictive models for the risk of obesity, altered WC and high BF% adjusted by age, ethnicity and sex. The predictive model for the risk of obesity (BMI Z-score >+2SD), altered WC (p > 80) and high BF% (boys ≥20% and girls ≥25%), suggested no interaction of the two loci. Single locus models (model with the smallest number of attributes) showed the highest cross validation consistency (CVC) and nearby the highest accuracy (Table 4).

Discussion

In the present study, we explored the association of the SNP *IRX3* rs3751723 with anthropometric characteristics associated with adiposity and potential relationships with the SNP *FTO* rs9939609 in a population of Brazilian children and adolescents. To the best of our knowledge, this study is the first to report the association of the *IRX3* gene with adiposity measurements in this population. In our results, it was possible to verify that *IRX3* rs3751723 was associated with obesity and BF%, whereas *FTO* rs9939609 was associated with a higher BMI Z-Score, obesity and WC. No linkage disequilibrium was observed between the analysed SNPs, and there is no interaction effect of these SNPs on anthropometric evaluated parameters.

The prevalence of obesity grows alarmingly in Brazil [16]. According to the national school health survey conducted in 2015 [35], 31.5% of the youngsters were overweight and obese. Among the Brazilian regions the highest index was in the southern region (38.4%) [35]. An earlier study with the same population of the present study showed prevalence of overweight and obesity of 41.3% in boys and 38.9% in girls [22]. The growth is evident, since 43.8% of the participants in the present study were overweight and obese.

The functional role of *IRX3* in obesity remains undefined. A study of *IRX3* deficient mice showed a marked reduction in adiposity with lower fat deposits as well as a reduction in adipocyte size and darkening of white adipose tissue [10]. A recent study with 1777 young Chinese showed that 17 rare variants of this gene were

Table 3
Multiple logistic regression for obesity, altered waist circumference (WC) and high body fat percentage (BF%).

Genotypes	OR (95% CI) obesity	P	OR (95% CI) altered WC	P	OR (95% CI) High BF%	P
IRX3						
TT	1		1		1	
GT	1.77 (0.80–3.93)	0.158	1.34 (0.68–2.65)	0.388	1.73 (0.96–3.10)	0.065
GG	2.61(1.18–5.76)	0.017	1.55 (0.78–3.06)	0.207	2.24 (1.24–4.06)	0.007
Dominant						
TT	1		1		1	
GT + GG	1.62 (1.09–2.39)	0.016	1.21 (0.83–1.76)	0.315	1.42 (1.03–1.96)	0.032
Recessive						
TT + GT	1		1		1	
GG	2.15 (0.99–4.63)	0.051	1.44 (0.75–2.76)	0.281	1.96 (1.11–3.43)	0.019
FTO						
TT	1		1		1	
TA	1.12 (0.73–1.72)	0.599	1.49 (0.98–2.27)	0.062	1.09 (0.77–1.54)	0.594
AA	1.89 (1.06–3.73)	0.031	2.21 (1.25–3.89)	0.006	1.24 (0.75–2.06)	0.395
Dominant						
TT	1		1		1	
TA + AA	1.27 (0.85–1.90)	0.247	1.63 (1.09–2.43)	0.016	1.13 (0.81–1.56)	0.469
Recessive						
TT + TA	1		1		1	
AA	1.77 (1.05–3.00)	0.033	1.76 (1.06–2.92)	0.029	1.18 (0.71–1.89)	0.181
Interaction model						
FTO AA (recessive)	2.25 (1.11–4.54)	0.023	2.10 (1.08–4.06)	0.028	1.48 (0.79–2.73)	0.215
IRX3 GG (recessive)	1.77 (1.15–2.71)	0.009	1.29 (0.86–1.96)	0.214	1.52 (1.08–2.15)	0.017
FTO x *IRX3*	0.59 (0.20–1.73)	0.339	0.62 (0.23–1.85)	0.430	0.59 (0.22–1.54)	0.284

Obesity: >+2SD, altered WC: percentile >80, high body fat percentage (BF%): boys ≥20%, girls ≥25%.
IRX3 rs3751723; *FTO* rs9939609. 95% CI, 95% confidence interval; OR, odds ratio OR adjusted for sex, ethnicity and age.

Table 4
GMDR analysis for *IRX3* rs3751723 and *FTO* rs9939609 for obesity-related phenotypes.

Anthropometrics	Model	Testing accuracy (%)	CVC	OR (95% CI)	P
Obesity					
	IRX3	55.6%	10/10	1.78 (0.27–11.72)	0.441
	IRX3xFTO	56.4%	10/10	1.90 (0.28–12.83)	0.439
Altered WC					
	FTO	55.2%	10/10	2.00 (0.33–12.10)	0.389
	IRX3xFTO	51.8%	10/10	1.44 (0.25–8.20)	0.492
High BF%					
	IRX3	54.2%	10/10	1.62 (0.36–7.41)	0.363
	IRX3xFTO	54.5%	10/10	1.73 (0.38–7.74)	0.345

Obesity: >+2SD, altered WC: percentile >80, high body fat percentage (BF%): boys ≥20%, girls ≥25%.
95% CI, 95% confidence interval; OR, odds ratio; CVC, Cross-validation consistency.

associated with human obesity. In addition, most of these variants are highly conserved among several species, which implies functional importance of the IRX3 protein [36]. This may explain the influence of *IRX3* on obesity and BF% found in the present study, supporting the idea that *IRX3* participates in body mass composition regulation. In our study, we observed an association between increased risk for obesity and BF%, especially in patients with the GG genotype. A study carried out in Malaysia also found association of GG genotype with greater risk for obesity (OR = 1.72, 95%CI = 1.02–2.91) [37]. However, studies with the Indian population showed that the TT genotype (OR = 3.3; 95%CI = 1.8–3.6) was associated with a higher risk for obesity [38], with T allele more frequently observed among obese (15.4%) than in non-obese subjects (6.9%) [15]. Another study with 333 Chinese adolescents identified other variants of *IRX3* (rs8053360 and rs1126960) that were associated with birth weight, a higher BMI and metabolism of AST/ALT transaminases [13]. Such data support the idea that the *IRX3* gene may be associated with the development of obesity.

In previous studies [10,13,37–39], *IRX3* rs3751723 and *FTO* rs9939609 were associated with obesity-related phenotypes. In Brazil, only *FTO* gene polymorphisms were investigated. A study with 16 thousand children and adolescents showed association of the *FTO* SNP rs9939609 with higher BMI ($p = 4.7 \times 10^{-10}$) [20], the same was observed with BMI Z-Score (p = 0.036) in one study with children in southern Brazil [21], but no study addressed the *IRX3* gene in our country. In our evaluation of the combination of the *IRX3* rs3751723 and *FTO* rs9939609 genotypes, *IRX3* and *FTO* were independently associated with to obesity parameters. In contrast, a study with 480 adults of Indian origin analysed gene-gene interaction using GMDR, showing genotypic combinations of *IRX3* rs3751723, *FTO* rs9939609 and *FTO* rs17817449 SNPs interacting for obesity risk (OR = 3.5, 95%CI = 2.1–5.9, p < 0.001) [15]. The cause of this discrepancy with our results may be due to age, ethnic differences between populations, different linkage disequilibrium between *IRX3-FTO* gene variants and non-combination with *FTO* rs17817449 variant. In addition, these controversial results indicate the existence of other factors that may be related to the effect of *FTO* and its relation with neighboring genes. Landgraf et al. [40] showed that only lean children who carry risk haplotypes of *FTO* for obesity had a reduction in the expression of *IRX3*. This shows that there may be a relationship between genes and that the environmental context may interfere. Additionally, it is speculated that a set of factors such as lifestyle (alcohol consumption and fast-food intake) and genetic outlook appear to be important components to be mutually considered in population study designs of overweight obesity management programs [37]. In this sense, additional genetic susceptibility and gene expression studies are needed to clarify the responsible genes. Furthermore, the effect of the IRX3 protein on human metabolism needs to be resolved.

This study is the first to analyse a variant of the *IRX3* gene in a young Brazilian population; however, some limitations are recognised. The sample size is representative of this population, but some studies use larger sample sizes in this type of study. We conclude that the genetic variants rs3751723 and rs9939609 influence the characteristics of adiposity in a sample of Brazilian children and adolescents. Although the *FTO* gene has been identified in several association studies in diverse populations, its role in the development of obesity still needs to be clarified. This study showed that the effect of *IRX3* and *FTO* investigated SNPs are independent predictors of adiposity parameters in this population. Additional research is needed to clarify the interaction between these genes.

Conflicts of interest

The authors declare that they have no conflict of interest.

Authors' contributions

P. F. T.: data collection, data analyses, data interpretation and manuscript writing. E. K.; C. P. R. and S. M.: data collection and genotyping. A. R. M. V. and J. A. M.: study design, data interpretation and manuscript writing. M. F.: study design, data analyses, data interpretation and manuscript writing. All authors contributed to the revision of the manuscript and approved the final manuscript.

Funding

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Coordination of Improvement of Higher Level Personnel (CAPES, Brazil). This study has been completed in partnership with the University of Navarra, Center of Biomedical Research in Physiopathology of Obesity and Nutrition (CIBERobn) and IMDEA Food, Madrid.

Ethical statement

The study protocol was approved by the Research Ethics Committee of the University of Santa Cruz do Sul (UNISC) under number 714 216/14 and by the Federal University of Health Sciences of Porto Alegre (UFCSPA) under number 995 205/15.

Acknowledgements

The authors thank all volunteers who participated in this study, the Biotechnology and Genetics Laboratory of UNISC and Molecular Biology Laboratory of IMDEA Food.

References

[1] González-Muniesa P, Mártinez-González MA, Hu FB, Després JP, Matsuzawa Y, Loos RJF, et al. Obesity. Nat Rev Dis Primers 2017;3:17034, http://dx.doi.org/10.1038/nrdp.2017.34.

[2] Kopelman PG. Obesity as a medical problem. Nature 2000;404(6778):635–43, http://dx.doi.org/10.1038/35007508.
[3] Vanlancker T, Schaubroeck E, Vyncke K, Cadenas-Sanchez C, Breidenassel C, González-Gross M, et al. Comparison of definitions for the metabolic syndrome in adolescents. The HELENA study. Eur J Pediatr 2017;176(2):241–52, http://dx.doi.org/10.1007/s00431-016-2831-6.
[4] Lakshman R, Elks CE, Ong KK. Childhood obesity. Circulation 2012;126(14):1770–9, http://dx.doi.org/10.1161/CIRCULATIONAHA.111.047738.
[5] Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518(7538):197–206, http://dx.doi.org/10.1038/nature14177.
[6] Hägg S, Ganna A, Van Der Laan SW, Esko T, Pers TH, Locke AE, et al. Gene-based meta-analysis of genome-wide association studies implicates new loci involved in obesity. Hum Mol Genet 2015;4(23):6849–60, http://dx.doi.org/10.1093/hmg/ddv379.
[7] Felix JF, Bradfield JP, Monnereau C, van der Valk RJ, Stergiakouli E, Chesi A, et al. Genome-wide association analysis identifies three new susceptibility loci for childhood body mass index. Hum Mol Genet 2016;25(2):389–403, http://dx.doi.org/10.1093/hmg/ddv472.
[8] Church C, Moir L, McMurray F, Girard C, Banks GT, Teboul L, et al. Overexpression of Fto leads to increased food intake and results in obesity. Nat Genet 2010;42(12):1086–92, http://dx.doi.org/10.1038/ng.713.
[9] Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, Rüther U. Inactivation of the Fto gene protects from obesity. Nature 2009;458(7240):894–8, http://dx.doi.org/10.1038/nature07848.
[10] Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gómez-Marín C, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature 2014;507(7492):371–5, http://dx.doi.org/10.1038/nature13138.
[11] National Center for Biotechnology Information, NCBI. Available in: https://www.ncbi.nlm.nih.gov/gene/79191.
[12] Gaborit N, Sakuma R, Wylie JN, Kim KH, Zhang SS, Hui CC, et al. Cooperative and antagonistic roles for Irx3 and Irx5 in cardiac morphogenesis and postnatal physiology. Development 2012;139:4007–19, http://dx.doi.org/10.1242/dev.081703.
[13] Liu C, Chu C, Zhang J, Wu D, Xu D, Li P. *IRX3* is a genetic modifier for birth weight, adolescent obesity and transaminase metabolism. Pediatr Obes 2018;13(3):141–8, http://dx.doi.org/10.1111/ijpo.12214.
[14] Ragvin A, Moro E, Fredman D, Navratilova P, Drivenes Ø, Engström PG, et al. Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to HHEX, SOX4, and IRX3. Proc Natl Acad Sci U S A 2010;107:775–80, http://dx.doi.org/10.1073/pnas.0911591107.
[15] Srivastava A, Mittal B, Prakash J, Srivastava P, Srivastava N, Srivastava N. A multianalytical approach to evaluate the association of 55 SNPs in 28 genes with obesity risk in North Indian adults. Am J Hum Biol 2017;29(2), http://dx.doi.org/10.1002/ajhb.22923.
[16] BRASIL, Ministério da Saúde. Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico. VIGITEL Brasil. 2014. http://www.abeso.org.br/uploads/downloads/72/553a243c4b9f3.pdf.
[17] Todendi PF, Klinger EI, Geraldo ACR, Brixner L, Reuter CP, Lindenau JDR, et al. Genetic risk score based on fat mass and obesity-associated, transmembrane protein 18 and fibronectin type III domain containing 5 polymorphisms is associated with anthropometric characteristics in South Brazilian children and adolescents. Br J Nutr 2018;12:1–7, http://dx.doi.org/10.1017/S0007114518002738.
[18] Santos HC, Horimoto AV, Tarazona-Santos E, Rodrigues-Soares F, Barreto ML, Horta BL, et al. A minimum set of ancestry informative markers for determining admixture proportions in a mixed American population: the Brazilian set. Eur J Hum Genet 2016;24(5):725–31, http://dx.doi.org/10.1038/ejhg.2015.187.
[19] Zembrzuski VM, Callegari-Jacques SM, Hutz MH. Application of an African ancestry index as a genomic control approach in a Brazilian population. Ann Hum Genet 2006;70:822–8, http://dx.doi.org/10.1111/j.1469-1809.2006.00270.x.
[20] Qi Q, Downer MK, Kilpeläinen TO, Taal HR, Barton SJ, Ntalla I, et al. Dietary intake, *FTO*. Genetic variants, and adiposity: a combined analysis of over 16,000 children and adolescents. Diabetes 2015;64(7):2467–76, http://dx.doi.org/10.2337/db14-1629.
[21] da Silva CF, Zandoná MR, Vitolo MR, Campagnolo PD, Rotta LN, Almeida S, et al. Association between a frequent variant of the *FTO* gene and anthropometric phenotypes in Brazilian children. BMC Med Genet 2013;14:34, http://dx.doi.org/10.1186/1471-2350-14-34.
[22] Reis LN, Renner JDP, Reuter CP, Horta JA, Paiva DN, Valim ARM, et al. Hipe-ruricemia está associada com baixos níveis de aptidão cardiorrespiratória e excesso de peso em escolares. J Pediat 2017;93(5):538–43, http://dx.doi.org/10.1016/j.jped.2016.11.011.
[23] Christensen LB. Experimental methodology. Boston: Allyn/Bacon; 1980.
[24] Santos SA. Historical roots of the whitening of Brazil. Latin Am Perspect 2002;122(29):61–82.
[25] Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 2003;100(1):177–82, http://dx.doi.org/10.1073/pnas.0126614100.
[26] Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ 2007;85(9):660–7.
[27] Taylor RW, Jones IE, Williams SM, Goulding A. Evaluation of waist circumference, waist-to-hip ratio, and the conicity index as screening tools for high trunk fat mass, as measured by dual-energy X-ray absorptiometry, in children aged 3–19 y. Am J Clin Nutr 2000;72:490–5, http://dx.doi.org/10.1093/ajcn/72.2.490.
[28] Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. Hum Biol 1988;60(5):709–23.
[29] Lohman TG. The use of skinfold to estimate body fatness on children and youth. J Phys Educ Recreat Dance 1987;58:98–103, http://dx.doi.org/10.1080/07303084.1987.10604383.
[30] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499–502.
[31] (NHLBI (National Heart, Lung, and Blood Institute). NHLBI Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents. NHI Publication; 2012, 12-7486A.
[32] ADA. (American Diabetes Association). Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care 2018;41(Suppl 1):S13–27, http://dx.doi.org/10.2337/dc18-S002.
[33] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
[34] Barrett JC, Fry B, Maller J, Daly MJ. Haploview analysis and visualisation of LD and haplotype maps. Bioinformatics 2005;21(2):263–5, http://dx.doi.org/10.1093/bioinformatics/bth457.
[35] IBGE. Pesquisa Nacional de Saúde do Escolar. Rio de Janeiro: IBGE; 2015. https://biblioteca.ibge.gov.br/visualizacao/livros/liv97870.pdf.
[36] Zou Y, Lu P, Shi P, Liu W, Yang M, Zhao S, et al. *IRX3* Promotes the browning of white adipocytes and its rare variants are associated with human obesity risk. EBioMedicine 2017;24:64–75, http://dx.doi.org/10.1016/j.ebiom.2017.09.010.
[37] Chong ETJ, Aziz NFA, Lee PC. Association of *IRX3* rs3751723 polymorphism with the risk of overweight and obesity: case-control study and meta-analysis. Meta Gene 2018;16:50–6. https://doi.org/10.1016/j.mgene.2018.01.007.
[38] Srivastava A, Mittal B, Prakash J, Srivastava P, Srivastava N, Srivastava N. Association of FTO and IRX3 genetic variants to obesity risk in north India. Ann Hum Biol 2016;43(5):451–6, http://dx.doi.org/10.3109/03014460.2015.1103902.
[39] Yang Q, Xiao T, Guo J, Su Z. Complex relationship between obesity and the fat mass and obesity locus. Int J Biol Sci 2017;13(5):615–29, http://dx.doi.org/10.7150/ijbs.17051.
[40] Landgraf K, Scholz M, Kovacs P, Kiess W, Körner A. FTO obesity risk variants are linked to adipocyte IRX3 expression and BMI of children – relevance of FTO variants to defend body weight in lean children? PLoS One 2016;11(8):e0161739, http://dx.doi.org/10.1371/journal.pone.0161739.