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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

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Abstract

The prevalence of childhood obesity has increased worldwide. Although it is considered a polygenic inheritance disease, little is known about its susceptibility when the additive effect is considered. The aim of this study is to investigate whether the genetic risk score (GRS) based on previously associated obesity polymorphisms (SNP) rs9939609 (fat mass and obesity-associated (*FTO*)), rs6548238 (transmembrane protein 18 (*TMEM18*)) and rs16835198 (fibronectin type III domain containing 5 (*FNDC5*)) could serve as a predictor for anthropometric characteristics in a sample of Brazilian children and adolescents. This is a cross-sectional study with 1471 children and adolescents aged 6–17 years. BMI, waist circumference (WC) and percentage of body fat and metabolic parameters were verified. In all, three SNP were genotyped by TaqMan™ allelic discrimination. The metabolic and anthropometric parameters were compared between the genotypes, and the unweighted and weighted GRS (GRS and wGRS, respectively) were created to test the additive effect of these genetic polymorphisms on anthropometric parameters. The prevalence of overweight plus obesity was 41 %. Significant associations were identified for *FTO* rs9939609, *TMEM18* rs6548238 and *FNDC5* rs16835198 and for GRS and wGRS with anthropometric phenotypes. The higher score of wGRS was associated with obesity (OR: 2·65, 95 % CI 1·40, 5·04, $P = 0·003$) and with greater WC (OR: 2·91, 95 % CI 1·57, 5·40, $P = 0·001$). Our results suggest that these genetic variants contribute to obesity susceptibility in children and adolescents and reinforce the idea that the additive effect may be useful to elucidate the genetic component of obesity.

Key words: **Obesity: Children: Adolescents: Genetic variants**

The prevalence of childhood obesity has increased substantially worldwide and has become a public health problem, particularly over the past two decades[1]. Alarming levels of obesity have been observed among children, with the prevalence of overweight and obesity varying significantly between different ethnic groups, sexes and socio-economic categories[1]. This complex phenotype results from the interaction of multiple genetic and environmental factors that influence BMI, with an estimated heritability ranging from 40 to 70 %[2].

Common obesity has polygenic inheritance, and genome-wide association studies (GWAS) and gene candidate studies have detected several associations between SNP and common diseases, including obesity[3]. These association studies identify genetic risk for obesity, which influence the development of obesity and accelerate the weight gain in infancy[3]. GWAS in populations of European descent identified more than ninety-seven genetic loci associated with obesity that belong to different pathways, such as insulin secretion, adipogenesis, energy

Abbreviations: BF%, body fat percentage; *FNDC5*, fibronectin type III domain containing 5; *FTO*, fat mass and obesity-associated; GRS, genetic risk score; *TMEM18*, transmembrane protein 18; WC, waist circumference; wGRS, weighted genetic risk score.

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metabolism and central nervous system (CNS)[4]. Using this approach, two new candidate genes related to obesity were identified: transmembrane protein 18 (*TMEM18*) and the fat mass and obesity-associated (*FTO*) gene, suggesting that they are responsible for the regulation of body weight, in part, through their actions in the CNS and adipose tissue[5]. Through a gene candidate approach, another gene was recently discovered: fibronectin type III domain containing 5 (*FNDC5*)[6] responsible for encoding the irisin protein, which can modulate lipid metabolism in adipose tissue, suggesting a possible role in energy homoeostasis[7].

Despite great improvements in the discovery of new obesity-related genes, little is known about susceptibility when the additive effects of these genetic variants are considered, which are important components of the genetic architecture of obesity. This study examined three genes that play a role in the regulation of energy homoeostasis. *FTO* and *TMEM18* genes were replicated in previous studies with children and adolescents[3,8], and *FNDC5* may provide new insights into biological factors that contribute to the development of obesity[7]. Therefore, the study aims to investigate whether the genetic risk score (GRS) based on previously associated obesity SNP rs9939609 (*FTO*), rs6548238 (*TMEM18*) and rs16835198 (*FNDC5*) could serve as a predictor for anthropometric characteristics in a sample of Brazilian children and adolescents.

Methods

Sample

This cross-sectional study comprised 1471 children and adolescents aged 6–17 years, 56 % of whom were female. The students were invited to participate in the study through informed consent signed by parents or guardians. 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 the Federal University of Health Sciences of Porto Alegre under number 995 205/15. The participants were consecutively recruited between March 2014 and December 2015 among students from the public and municipal network of Santa Cruz do Sul, Rio Grande do Sul, Brazil. The sample was selected by convenience and was included in the study of healthy subjects, who did not have restrictions for blood collections. The population from Southern Brazil is ethnic mixed[9]; thus, the ethnicity determination was made according to Parra *et al.*[10], based on an evaluation of the following phenotypic characteristics: skin colour in the medial part of the arm; colour and texture of hair; and the shape of the nose and lips. In this sense, 75 % of the subjects were determined as European descendants.

Anthropometric measures

Body weight and height (coupled stadiometer) were measured using a Welmy balance (Welmy: 15416). BMI was defined by the following formula: BMI = weight/height^2 (kg/m^2). BMI *Z*-score was classified by World Health Organization[11] according to sex and age, considering low/normal weight (<1SD), overweight (>1SD) and obesity (>2SD). Waist circumference (WC) was determined using inelastic tape, with reference to the narrowest part of the trunk between the ribs and the iliac crest and hip at the level of the greater trochanter. A normal WC was classified as percentile ≤80 and obesity as percentile >80, according to previously established criteria[12]. The Lange® compass (Beta Technology Incorporated) was used to measure body fat percentage (BF%), using measurements of triceps and subscapular skinfolds. Subsequently, the equation of slaughter was applied and the data were classified according to the criteria established by Lonman's date, as mentioned by Heyward & Stolarczyk[13], into two BF% categories: (1) very low, low and excellent and (2) moderately high, high and very high.

Biochemical analyses

Blood samples were collected by venepuncture after a 12-h fast. Total cholesterol (TC), HDL-cholesterol, TAG and glucose were measured using Kovalent commercial kits (BioSys Ltda) with a Miura One equipment (ISE). LDL-cholesterol was calculated using the Friedewald equation[14].

Genotyping

EDTA-anticoagulated whole blood was used for DNA extraction by the salting out method[15]. DNA was then quantified using a NanoDrop 2000c spectrophotometer unit (Thermo Scientific). The genotyping of polymorphisms *TMEM18* rs6548238, *FTO* rs9939609 and *FNDC5* rs16835198 was performed using Taqman™ allelic discrimination assays (Applied Biosystems) in StepOne Plus® equipment, according to the manufacturer's instructions. TaqMan™ assays C__29311887_10 (rs6548238), C__30090620_10 (rs9939609) and C__34204885_10 (rs16835198) and Master Mix PCR Universal were purchased from Applied Biosystems.

Statistical analysis

The Kolmogorov–Smirnov test was used to verify the normality of the observed variables. Values expressed as the mean and standard deviation were used to describe continuous variables. Allele frequencies were estimated by gene counting. The allelic and genotype distribution between groups and Hardy–Weinberg equilibrium were tested using the χ^2 test. A risk score approach was used to evaluate the combined effects of SNP on anthropometric characteristics. The unweighted GRS was constructed using a genotypic score based on the number of unfavourable alleles (those associated with higher levels of BMI *Z*-score and WC) that were carried by each subject for each of the three SNP[16]. Risk alleles were those previously associated with obesity or obesity-related phenotypes in the literature and according to the data of the present study. For SNP rs6548238, rs9939609 and rs16835198, the C, A and T alleles were considered risk alleles, respectively. Weighted GRS (wGRS) was generated by weighting each allele using the natural log of the published OR reported in UK Biobank GWAS results (https://sites.google.com/broadinstitute.org/ukbbgwasresults/). Multiple linear regression analyses were used to adjust

anthropometrics variables for age, sex and ethnicity. Mean adjusted variables were compared among genotypes and GRS (GRS and wGRS) by ANOVA. Multiple logistic regression analysis was carried out to estimate the OR with 95 % CI in order to assess genetic risk factors (genotypes, GRS and wGRS) for obesity parameters, as well as to control for confounding factors. A *P*-value <0·05 was considered statistically significant. Statistical analyses were performed using SPSS 23.0 for Windows.

Justification of sample size

The number of students needed to have a representative sample of the city was calculated using the total number of students enrolled using data provided by the sixth Regional Educational Coordinator of Rio Grande do Sul and the Municipal Department of Education of Santa Cruz do Sul. For this purpose, size was calculated using the Nea Research Division[17] formula, suggesting that for 20 450 students enrolled the sample should consist of at least 392 students with a 5 % error. The analysed sample (1471 subjects) has a statistical power above 90 % to detect an OR of 1·5, with a significance level of 5 % with 95 % for SNP with a minor allele frequency of at least 15 %.

Results

Descriptive characteristics of the subjects of this study are presented in Table 1, which comprises 76·5 % of adolescents and 56·2 % of female subjects, with a mean age of 11·9 (SD 2·8) years. According to the anthropometric characteristics, 23·0 % were overweight, 18·0 % were obese, 21·7 % had high WC and 35·4 % had high BF% moderately, high and very high.

The genotypic proportions were in Hardy–Weinberg equilibrium for the three polymorphisms. Frequencies of the minor alleles were 37 % for rs9939609/A (*FTO*), 14 % for rs6548238/T (*TMEM18*) and 35 % for rs16835198/T (*FNDC5*). A comparison of the BMI *Z*-score, WC, BF% and metabolic parameters among genotypes is shown in Table 2. *FTO* rs9939609 was associated with higher BMI *Z*-score ($P=0·005$), WC ($P=0·035$) and TAG ($P=0·007$), whereas *TMEM18* rs6548238 was associated with BMI *Z*-score ($P=0·008$), WC ($P=0·009$) and BF% ($P=0·024$). For the rs16835198 at *FNDC5* gene, homozygotes TT presented a higher BMI *Z*-score ($P=0·005$) and WC ($P=0·009$).

Table 3 shows the means of BMI *Z*-score, WC and BF% in relation to GRS groups. The means of BMI *Z*-score and WC were found to be higher as the number of risk alleles increased ($P=0·015$, $P<0·001$, respectively). Although a greater tendency of the means of BF% in relation to the GRS groups was observed, this association was not significant.

Fig. 1(a) and (b) show the associations of WC categories (normal percentile ≤80 and high percentile >80) and BMI *Z*-score (low weight/normal: <1SD; overweight: >1SD; obesity: >2SD) with GRS. We observed that as the number of risk alleles increased, the percentage of individuals with altered WC ($P=0·001$) and with obesity ($P=0·017$) also increased. Among individuals with no risk alleles, none presented altered WC, whereas among those with six risk alleles, 44 % presented WC percentile >80 (Fig. 1(a)). Regarding BMI *Z*-score, 17 % of individuals without risk alleles presented BMI *Z*-score in the overweight range and none was obese. For those with six risk alleles, 24 % were overweight and 40 % were obese (Fig. 1(b)). Association effects of the GRS and wGRS were tested for obesity (BMI *Z*-score >2SD) and altered WC (percentile >80). The higher score of wGRS was associated with obesity (OR: 2·65, 95 % CI 1·40, 5·04, $P=0·003$) and with greater WC (OR: 2·91, 95 % CI 1·57, 5·40, $P=0·001$) (Table 4).

Table 1. Descriptive characteristics of the subjects (*n* 1471)
(Mean values and standard deviations; numbers and percentages)

Variables	*n*	%	Mean	SD
Sex				
Female	826	56·2		
Male	645	43·8		
Age (years)			11·9	2·8
Age range*				
Children	345	23·5		
Adolescents	1126	76·5		
BMI *Z*-score†			0·78	1·3
Low/normal weight	867	58·9		
Overweight	339	23·0		
Obesity	265	18·0		
WC‡			67·4	10·7
Normal	1152	78·3		
High	319	21·7		
BF%			20·8	7·6
Very low, low and excellent	950	64·6		
Moderately high, high and very high	520	35·4		

WC, waist circumference; BF%, body fat percentage.
* Age range: children 7–9 years, adolescents 10–17 years.
† BMI *Z*-score: low/normal weight <1SD, overweight >1SD, obesity >2SD.
‡ WC: normal percentile ≤80 and high percentile >80.

Table 2. Association between each SNP of fat mass and obesity-associated (*FTO*), transmembrane protein 18 (*TMEM18*) and fibronectin type III domain containing 5 (*FNDC5*) genes with anthropometric characteristics and metabolic parameters*
(Mean values and standard deviations)

		BMI *Z*-score		WC		BF%		TC (mmol/l)		LDL-C (mmol/l)		HDL-C (mmol/l)		TAG (mmol/l)		Glucose (mmol/l)	
Genes	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FTO rs9939609																	
TT	583	0·70	1·26	66·7	9·8	20·7	7·3	4·10	0·86	2·11	0·73	1·62	0·30	0·79	0·37	4·93	0·60
TA	689	0·80	1·28	67·5	10·7	20·7	7·7	4·11	0·82	2·12	0·70	1·60	0·30	0·85	0·42	5·05	1·90
AA	198	0·96	1·33	69·1	12·8	21·3	8·1	4·05	0·90	2·07	0·74	1·58	0·31	0·86	0·50	4·91	0·60
P		0·005		0·035		0·380		0·755		0·748		0·139		0·007		0·208	
TMEM18 rs6548238																	
CC	1083	0·85	1·28	67·9	10·9	21·1	7·7	4·11	0·85	2·12	0·71	1·61	0·31	0·84	0·43	5·00	1·52
CT	355	0·60	1·28	66·4	10·0	20·1	7·2	4·07	0·84	2·09	0·75	1·61	0·27	0·80	0·36	5·00	0·86
TT	33	0·50	0·99	64·0	8·7	19·5	6·4	3·97	0·95	2·11	0·77	1·52	0·25	0·74	0·39	4·85	0·50
P		0·008		0·009		0·024		0·441		0·760		0·290		0·069		0·855	
FNDC5 rs16835198																	
GG	624	0·82	1·25	67·8	10·5	20·9	7·6	4·10	0·87	2·14	0·73	1·60	0·30	0·82	0·44	5·00	1·94
GT	658	0·71	1·26	66·5	9·9	20·3	7·2	4·12	0·83	2·10	0·71	1·63	0·30	0·83	0·40	5·00	0·72
TT	189	0·91	1·42	68·9	13·4	22·0	8·6	4·03	0·81	2·06	0·70	1·60	0·31	0·80	0·40	4·92	0·60
P		0·005		0·009		0·100		0·280		0·276		0·078		0·291		0·836	

WC, waist circumference (percentile); BF%, body fat percentage; TC, total cholesterol.
* ANOVA, adjusted by sex, age and ethnicity.

Table 3. Anthropometric characteristics according unweighted and weighted genetic risk score of the three SNP in fat mass and obesity-associated (*FTO*), transmembrane protein 18 (*TMEM18*) and fibronectin type III domain containing 5 (*FNDC5*) genes with anthropometric characteristics and metabolic parameters
(Mean values and standard deviations)

		BMI *Z*-score		WC (cm)		BF%	
	n	Mean	SD	Mean	SD	Mean	SD
GRS†							
No risk allele	6	0·30	0·83	59·9	5·6	19·6	6·0
01	83	0·76	1·15	68·0	10·4	21·6	7·6
02	309	0·65	1·29	66·4	10·0	20·0	7·2
03	521	0·77	1·22	67·3	9·8	20·7	7·2
04	405	0·80	1·33	67·9	10·6	20·9	7·9
05	122	1·01	1·37	69·6	13·0	21·9	8·6
06	25	1·37	1·34	75·3	19·4	23·8	8·3
P		0·015		<0·001		0·177	
wGRS							
0	6	0·30	0·84	59·9	5·6	19·6	6·0
w01 (0·01–0·09)‡	174	0·60	1·21	66·8	9·4	20·5	7·0
w02 (0·10–0·14)‡	586	0·71	1·29	66·4	10·1	20·5	7·3
w03 (0·15–0·19)‡	526	0·86	1·26	68·1	10·7	21·1	7·8
w04 (0·20–0·24)‡	96	0·93	1·35	68·0	11·4	21·3	8·3
w05 (0·25–0·27)‡	83	1·04	1·36	71·0	14·9	21·7	8·1
P		0·021		0·001		0·506	

WC, waist circumference; BF%, body fat percentage; GRS, genetic risk score; wGRS, weight genetic risk score.
* Association analysis adjusted by sex, age and ethnicity.
† GRS: no risk allele, 01 – one risk allele, 02 – two risk alleles, 03 – three risk alleles, 04 – four risk alleles, 05 – five risk alleles and 06 – six risk alleles.
‡ Weighted value: number of alleles risk multiplied by the natural log of the published OR.

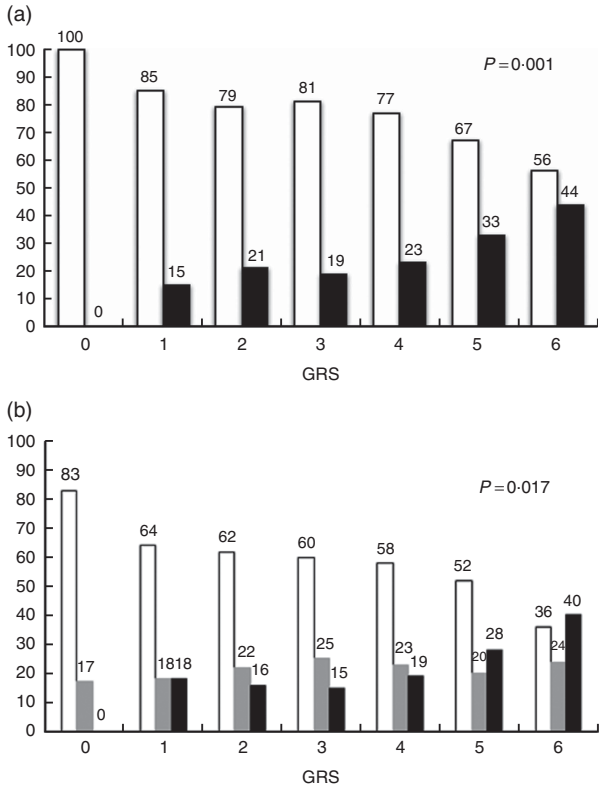

Fig. 1. (a) Association of categories of waist circumference (WC) with genetic risk score (GRS). □, WC percentile <80; ■, WC percentile ≥80. (b) Association of BMI *Z*-score categories with GRS. *Z*-score: low/normal weight, <1SD (□); overweight, >1SD (▨); obesity, >2SD (■). GRS: 0, no risk allele; 1, one risk allele; 2, two risk alleles; 3, three risk alleles; 4, four risk alleles; 5, five risk alleles; 6, six risk alleles.

Discussion

The objective of this study was to investigate whether there is an additive effect of SNP rs9939609 (*FTO*), rs6548238 (*TMEM18*) and rs16835198 (*FNDC5*) on anthropometric characteristics in Brazilian children and adolescents. The comparison between the anthropometric variables evaluated among genotypes of each SNP replicated the significant associations that have already been observed in other studies on obesity, mainly WC and BMI. The main finding of this study is the cumulative risk effect for obesity and altered WC according to

Table 4. Multiple logistic regression for obesity (BMI *Z*-score >2SD) and for high waist circumference (WC percentile >80) according unweighted genetic risk score (GRS) and weighted genetic risk score (wGRS) of the three SNP in fat mass and obesity-associated (*FTO*), transmembrane protein 18 (*TMEM18*) and fibronectin type III domain containing 5 (*FNDC5*) genes*
(Odds ratios and 95 % confidence intervals)

	Obesity			WC percentile >80		
	OR	95 % CI	*P*	OR	95 % CI	*P*
GRS†						
0–1	1			1		
2	0·80	0·42, 1·52	0·497	1·60	0·82, 3·14	0·170
3	0·81	0·44, 1·50	0·509	1·44	0·75, 2·76	0·275
4	1·03	0·55, 1·91	0·927	1·85	0·96, 3·56	0·067
5	1·71	0·85, 3·43	0·131	2·97	1·44, 6·13	0·003
6	3·38	1·25, 9·17	0·017	4·99	1·82, 13·70	0·002
wGRS						
w0–1	1			1		
w02	1·11	0·68, 1·80	0·676	1·34	0·84, 2·12	0·218
w03	1·27	0·78, 2·07	0·328	1·74	1·10, 2·76	0·018
w04	2·15	1·14, 4·06	0·018	1·92	1·03, 3·58	0·040
w05	2·65	1·40, 5·04	0·003	2·91	1·57, 5·40	0·001

* OR adjusted for sex, age and ethnicity.
† GRS: 0–1 no risk allele or one risk allele, 02 – two risk alleles, 03 – three risk alleles, 04 – four risk alleles, 05 – five risk alleles, 06 – six risk alleles.

the GRS created to evaluate the combined effects of the three SNP. The BF% showed no association with the GRS; supposedly other genes are associated with this adiposity variable.

Our data show that the allelic frequencies found are similar to previous studies[8,18,19]. Minor allelic frequencies of rs9939609/A (*FTO*) and rs6548238/T (*TMEM18*) were 35 and 15 %, respectively, in other studies with Brazilian population[8,18]. The rs16835198/T allele (*FNDC5*) has not yet been genotyped in Brazilian populations, but a study with European offspring shows a frequency of 35 % for the minor allele[19], confirming the same value found in our study.

Variants of the *FTO* and *TMEM18* genes were initially associated with obesity in adults[20,21]; the *FNDC5* gene variant appears to influence glycolipid metabolism in overweight/obese adult subjects[22]. In addition, researchers have recently investigated the effect of these variants on the risk of obesity as early as childhood and adolescence[3,8]. Early obesity, particularly in children, is highly predictive of obesity in adulthood, especially when one (or both) of the parents is obese[23]. This early obesity is one of the determinants of cardiometabolic risk in adulthood[24].

The *FTO* gene is involved in the stimulation of food intake and is highly expressed in the human hypothalamus, pituitary gland and adrenal glands, suggesting a potential role in the hypothalamic–pituitary–adrenal axis and implying the regulation of body weight. Several potentially functional SNP at the *FTO* locus are highly associated with the early onset of obesity and severe obesity in European populations[25]. The most studied SNP of this gene, rs9939609, was evaluated in this study and was consistently associated with higher values of BMI *Z*-score, WC and TAG ($P < 0·05$) in children and adolescents. Other studies performed with this same age group and populations of European and Asian and Amerindian origin also show an association of this SNP rs9939609 with higher means of BMI *Z*-score, WC and TAG ($P < 0·05$)[20,26,27].

The *TMEM18* gene is expressed or known to act on the CNS, although it also exhibits peripheral functions related to adipose tissue[28]. To better understand the relation of this gene to obesity, this study examined the relationship between variant rs6548238 and anthropometric characteristics. We observed that C allele carriers presented higher BMI *Z*-score, WC and BF% values ($P < 0·05$). Other studies involving children and adolescents have also shown an association of this variant with BMI *Z*-score, WC and BF% in populations of Mexican[29], European[30] and New Zealand origin[31].

Since its discovery in 2012, the irisin protein encoded by the *FNDC5* gene has attracted interest as a potential health mediator, promoting effects on physical exercise. It is considered to be a novel hormone-like myokine released by skeletal muscle during exercise to improve obesity and glucose dysfunction, stimulating the darkening of white adipose tissue[6]. Evidence indicates that irisin is expressed robustly, not only in skeletal muscle[6] but also in various regions of brain tissue[32]. To the best of our knowledge, there is only one study with adults and a population of Chinese origin that associated the rs16835198 SNP of the *FNDC5* gene with glycolipid metabolism in overweight/obese subjects[22]. However, this study is the first to observe the relationship of this SNP with higher means of BMI *Z*-score and WC ($P < 0·005$) in children and adolescents.

Although GWAS have identified a significant number of SNP associated with many complex human characteristics, the susceptibility loci identified to date may account for only a small fraction of the genetic risk[33]. Although several factors such as rare genetic variants, structural variations, epigenetic modifications and gene–environment interactions may contribute to 'missing heritability'[34], the additive effect of several SNP has been poorly explored and becomes an important component of

multifactorial genetic diseases owing to the complexity of biological systems[35]. In this sense, numerous studies have already evaluated the SNP studied in this research with obesity, but very few studies have evaluated the simultaneous effect of the presence of the risk alleles of each SNP.

Thus, it was observed in this study that the additive effect among the studied SNP was higher as the number of risk alleles of individuals with altered WC ($P<0{\cdot}001$) and with obesity increased ($P=0{\cdot}015$). In addition, our data showed that subjects with higher GRS are associated with higher risk of obesity and altered WC (Table 4). The association was significant both for unweighted GRS and wGRS. To date, only four studies on additive effects have shown an association of obesity and the rs9939609 SNP of the *FTO* gene, one study has shown an association with the variant rs6548238 of the *TMEM18* gene and no study has uncovered an association with the variant rs16835198 of the *FNDC5* gene.

A study with children and adolescents of European origin tested the combination of *ACE* (rs4646994), *FTO* (rs9939609), *MC4R* (rs17782313) and *PPARG* (rs1801282) SNP with obesity, showing influence of *MC4R* and *PPARG* with higher BMI[36]. Another study with an adult population of Indian origin evaluated fifty-five SNP in twenty-eight genes. However, the SNP *FTO* (rs9939609), iroquois homeobox 3 (*IRX3*) (rs3751723), transcription factor 7 like 2 (*TCF7L2*) (rs7903146) and *TMEM18* (rs6548238) were observed to be the main SNP that contribute to the risk of obesity linked to BMI[37]. A study of Indian adults tested the additive effect of SNP *FTO* (rs8050136, rs1421085, rs9939609, rs17817449) and *IRX3* (rs3751723) and observed the association of these genes with the risk for obesity[38]. Finally, a study of Chinese adults tested the additive effect among eleven variants of the *FTO* gene (among them rs9939609), *TSPAN8* and *TCF7L2*, and they were associated with type 2 diabetes mellitus and obesity[33].

Although *FTO* and *TMEM18* SNP were associated with obesity in many studies[4,8,20,30,31], some researchers found no association of the rs6548238 (*TMEM18*)[39] and rs16835198 (*FNDC5*)[40] variants with adiposity parameters. To the best of our knowledge, this is the first study to test the variant rs16835198 of the *FNDC5* gene in obese children and adolescents and to analyse its cumulative effect with other SNP. It is also the first study to analyse the cumulative effect of these three SNP in a sample composed of Brazilian children and adolescents. However, there were some limitations. Of all Brazilian regions, European immigrants predominantly populate the southern region. According to Parra *et al.*[10], in Brazil the colour determined by the physical evaluation is a weak predictor of genomic ancestry. This factor may limit this study, as the ancestry was self-declared and may not reflect the real ancestry of the research subjects. Sample size is representative of this population, although some studies use larger sample size in this type of study. Further, sufficient statistical power was allowed for our analyses, and these data can be incorporated into future meta-analyses.

In conclusion, three SNP analysed of the genes *FTO*, *TMEM18* and *FNDC5* were found to be associated with the susceptibility of developing greater BMI *Z*-score and WC in a sample composed of Brazilian children and adolescents. These findings are consistent with other studies that consistently associate these genes with obesity. Together with our results, these data corroborate the idea that the cumulative effect may be useful to understand the genetic component of obesity. The results of this study also suggest that this method may be an option for future studies, mainly in order to find the missing heritability.

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P. F. T. was involved in data collection, data analyses, data interpretation and manuscript writing. E. I. K., A. C. R. G. and L. B. contributed to data collection and genotyping. C. P. R. and A. R. M. V. contributed to study design, data interpretation and manuscript writing. J. D. R. L. and M. F. contributed to study design, data analyses, data interpretation and manuscript writing. All authors contributed to the revision of the manuscript and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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