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BIOMEDICAL SCIENCES

Investigation of associations of European, African, Amerindian genomic ancestries and *MC4R, FTO, FAIM2, BDNF* loci with obesity-related traits in Rio de Janeiro, Brazil

ALLAN SCHARF, FLÁVIA F. BEZERRA, VERÔNICA M. ZEMBRZUSKI, ANA CAROLINA P. DA FONSECA, LEONOR GUSMÃO & EDUARDO FAERSTEIN

Abstract: A complex web of causation is involved in adiposity, including environmental, social and genetic factors. We aimed to investigate associations between genetic factors such as ancestry and single nucleotide polymorphisms, and obesity-related traits in a sampled Brazilian population. A sample of 501 unrelated adults participating in 2013 at the longitudinal Pró-Saúde Study (EPS) in Rio de Janeiro, Brazil was selected. We analysed 46 AIM-InDels (insertion/deletion) as genetic ancestry markers and four single nucleotide polymorphisms located in the genes *MC4R* (rs17782313), *FTO* (rs9939609), *FAIM2* (rs7138803) and *BDNF* (rs4074134), previously described as associated with obesity. The selected obesity-related markers were anthropometric parameters such as body mass index, waist circumference and waist-to-hip ratio, and body composition measurements namely body fat percentage, android fat mass and gynoid fat mass. The sample showed greater European ancestry (57.20%), followed by African (28.80%) and lastly Amerindian (14%). Our results suggest that the rs4074134 (*BDNF*) CC genotype was directly associated with gynoid fat mass, whereas body fat percentage, android fat mass and the anthropometric parameters seem not to be associated with neither ancestry nor the four polymorphisms in this population sample, most likely due to a stronger role of social, behavioural and environmental determinants.

Key words: adiposity, ancestry, association study, Brazilian population, obesity.

INTRODUCTION

Obesity, a condition once rare, increased over time in incidence, and was finally identified as a health problem in the middle of the 19th century (Wells 2006). However, since 1980, the Body Mass Index (BMI = weight/height^2) has already increased by an average of 0.4 kg/m^2 per decade worldwide. In 2000, for the first time, the number of overweight adults surpassed those with a deficit (Finucane et al. 2011), and ten years later the worldwide prevalence of obesity has doubled. In 2016, the World Health Organization (WHO) published that 39% of adults were already overweight (BMI ≥ 25 kg/m^2) and 13% were obese (BMI ≥ 30 kg/m^2) (World Health Organization 2020). Low and middle income countries, largely located in Africa and the Americas, have registered a faster increase in the prevalence of overweight and obesity (Ng et al. 2014).

In Brazil, a national study called Surveillance of Risk and Protection Factors for Chronic Diseases by Telephone Survey identified that 55.40% of individuals aged 18 or over are overweight and 20.30% are obese. Prevalence of overweight increased from 42.60% in 2006 to

55.40% in 2019, being highest among men (57.10%) and adults aged 65 and over (59.80%). Prevalence of obesity was highest among women (21%) and also increased with age, reaching 20.90% among adults aged 65 and over. In Rio de Janeiro, the prevalence of obesity and overweight resemble the national average (Ministério da Saúde 2020).

Obesity is a complex condition influenced by genetic, behavioural, nutritional, social and environmental factors (Loos & Bouchard 2003, Velázquez-Fernández et al. 2017). The most common form of obesity appears not to follow a genetic inheritance model and is caused by an interaction of major effect environmental factors with multiple genetic variants with minor effect (Da Fonseca et al. 2017).

The role of genetics in susceptibility to obesity has been investigated since the early 2000s. The most accessible parameter for the assessment of genetic factors and obesity risk is the BMI, although other parameters (eg. waist circumference, waist-to-hip ratio and body fat percentage) have emerged given its association with obesity and some of its risk factors (World Health Organization 2008, Duclos 2016, Patnaik et al. 2017).

Genome wide association studies (GWAS) is a well-established and effective approach of identifying candidate risk loci in the human genome containing genetic variations associated with complex diseases or phenotypes (Patron et al. 2019). Wu et al. (2018) through a GWAS recently revealed 291 nominally obesity-associated genes in a Northern Han Chinese cohort and showed that these genes have an important role in different pathways, such as homeostasis, olfactory transduction and platelet production. Interestingly, several GWAS have been consistently identified candidate genes that may be causally linked to obesity, such as *MC4R*, *FTO*, *FAIM2* and *BDNF* (Hotta et al. 2009, Ng et al. 2010, Dorajoo et al. 2012).

Furthermore, there is also a possible association between ancestry, ethnicity and the clinical syndrome, showing the importance of better understanding the history of populations (Sabin et al. 2011, Waalen 2014, Pigeyre et al. 2016, Yu 2017, Qasim et al. 2018). This aspect may be even more relevant in admixed populations from well-differentiated groups (Grant et al. 2008, 2009, Ningombam et al. 2018). Obesity-related traits have been associated with the European, African or Native ancestries in different ethnic groups. It is interesting to note that some studies demonstrate that the estimates of admixture from an ancestral population are directly related to BMI, while others report an inverse association (Fernández et al. 2013). These discrepancies seem to be affected by ethnicity, environmental factors, gender and the number of ancestral populations considered in the study.

The genetic markers called "Ancestry Informative Markers (AIMs)" have a high differentiation between their allele frequencies in different or geographically distant ancestral populations. These markers are especially useful to infer the ancestral origin of a given individual and to estimate the proportions of different lineal origins of admixed populations (Shriver et al. 1997). In studies of genetic association with obesity risk, ancestry may be a confounder. It is vital to use AIMs as a control variable along with the single nucleotide polymorphisms described as related to obesity in populations that may be under stratification, in order to avoid the observation of erroneous associations.

The aim of this study was to investigate whether *FTO*, *MC4R*, *FAIM2* and *BDNF* variants/loci identified in GWAS as well as AIMs contribute to the obesity-related traits in a Brazilian population.

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

MATERIALS AND METHODS

Study design and population

This is a cross-sectional study conducted in working-age unrelated individuals from a cohort study of university civil servants in Rio de Janeiro, the Pró-Saúde Study (EPS). The EPS is a longitudinal prospective study of non-faculty public agents at the State University of Rio de Janeiro, Brazil, which focus on the investigation of health-related social and behavioural determinants (Faerstein et al. 2005). Four waves of data collection have been conducted among 3 253 participants (1999, 2001, 2006, and 2012). During the fourth data collection phase, a sample of 520 participants was selected to perform additional assessments absent in the first three waves, including body composition by dual energy X-ray absorption (DXA) and blood collection for genetic ancestry analysis. This sample was randomly defined considering the proportions of the strata of gender, age (up to 50 years vs. 51 years or more) and level of education (up to complete high school vs. complete university or more) in the cohort population.

All samples were collected under written informed consent and the project was approved by the Research Ethics Committee of the Institute of Social Medicine of the State University of Rio de Janeiro (CAAE 0041.0.259.000-11 and 04452412.0.0000.5260). All procedures performed in this study involving human participants were in accordance with the ethical principles of the 2000 Helsinki Declaration of the World Medical Association (2013).

Samples and DNA extraction

The extraction and purification of leukocyte DNA from total blood samples was performed using the commercial kit (Puregene Blood Kit, Qiagen, Düsseldorf, Germany). DNA quality and integrity were evaluated via electrophoresis on 0.80% agarose gel. DNA samples were quantified by spectrophotometry in NanoDrop (Thermo Fisher Scientific, Carlsbad, CA, USA), aliquoted and stored at -80 °C until use.

Genotyping of 46 AIM-InDels and ancestry estimation

All samples were genotyped for 46 AIM-InDels by PCR and capillary electrophoresis according to the protocol described in Pereira et al. (2012). The 46 AIM-InDels system used was optimized to efficiently measure admixture proportions from four different continental origins (Africa, Europe, America and East Asia) in a single PCR-multiplex (Pereira et al. 2012).

The amplified products were analysed by capillary electrophoresis on an ABI 3500 (Applied Biosystems, Waltham, MA, USA). Genotyping was performed using the software GeneMapper ID-X v1.2 and/or GeneMapper ID v4.1 (Thermo Fisher Scientific).

Ancestry estimates of the individuals from the population sample were obtained using the software STRUCTURE v2.3.4 (Pritchard et al. 2000), with reference population data from 98 African (AFR), 147 European (EUR) and 51 Native (NAM) individuals from the HGDP-CEPH diversity panel as reference (Pereira et al. 2012).

The STRUCTURE analyses were carried out using 100 000 burnin steps, followed by 100 000 iterations Markov Chain Monte Carlo (MCMC). In the analysis, three clusters (K) were assumed, and to verify the consistency of the results, five independent runs were performed. These five runs were combined using the software CLUMPP v.1.1.222 (Jakobsson & Rosenberg 2007) to obtain

the best estimate of the cluster membership coefficients.

Genotyping of the four SNPs described in association to obesity

Four single nucleotide polymorphisms (SNPs) described in association to overweight/obesity factors were genotyped in the Brazilian population sample: rs17782313 (*MC4R*), rs9939609 (*FTO*), rs7138803 (*FAIM2*) and rs4074134 (*BDNF*).

The rs17782313 (*MC4R*) was the first locus in which mutations directly associated with dominant inherited human obesity were identified, and was the most investigated genetic cause of obesity before the era of GWAS (Huszar et al. 1997, Yeo et al. 1998). The rs17782313 polymorphism has been associated with increased BMI and waist circumference (Loos 2011), insulin resistance (Tschritter et al. 2011), diabetes mellitus type 2 and lipid intake (Khalilitehrani et al. 2015). In addition, the presence of the risk allele (C) has shown a relationship with uncontrolled food intake (Vega et al. 2016).

Described as one of the genetic factors that lead to the onset of obesity and its metabolic implications by the hedonic mechanism of food reward, the rs9939609 (*FTO*) increased expression may lead to obesity (Church et al. 2010). *FTO* associations with body fat mass and other obesity-related traits have been identified in different investigations (Dina et al. 2007, Frayling et al. 2007, Scuteri et al. 2007, Shabana & Hasnain 2015).

The rs7138803 (*FAIM2*) is an apoptosis-inhibiting gene apparently related to obesity. Epigenetic changes are important factors in helping develop obesity and it can help understand this process. Methylation levels at 8 sites in the gene promoter were significantly different between obese and lean individuals. This study provided the first evidence that the methylation levels of the *FAIM2* promoter are significantly associated with obesity (Wu et al. 2015).

The least investigated, rs4074134 (*BDNF*) has been explored by fewer studies, and most of them were in Asian populations. Han et al. (2013) found an association between this polymorphism and the reduction of BMI, WC, glucose, insulin and risk for type 2 diabetes in Chinese Han population. In a Japanese population, however, Hotta et al. (2009) observed that this genetic variation, as well as SNPs in *FAIM2* and *MC4R* genes, was marginally associated with obesity susceptibility. Through GWAS, seven SNPs located within or downstream of *BDNF* at 11p14, including rs4074134 (*BDNF*), were found to be associated with BMI and total mass (Thorleifsson et al. 2009).

These variants were determined through real-time polymerase chain reaction TaqMan® SNP genotyping assays (Thermo Fisher Scientific). Amplification was performed in a StepOne® Plus real-time PCR system (Thermo Fisher Scientific) and data analyses were carried out using StepOne software v2.3 (Thermo Fisher Scientific).

Obesity-related markers assessment

The total body mass, total fat mass, and android and gynoid fat masses (AFM and GFM respectively) were measured via DXA with iDXA Lunar equipment (GE Healthcare, Milwaukee, WI, USA) and enCORE 2008 software version 12.20 (iDXA GE Medical Systems enCORE Operator's Manual and Product Information). The body fat percentage (BF) was derived from the total mass and the total fat mass (total fat mass/total mass x100).

For the full-body examination, participants used light clothing and no accessories that could skew the measurements. They were placed in the supine position and asked to

remain immobile until the end of the procedure. The android region was defined by the software as the region from the top of the iliac crest to 20% of distance from chin to iliac crest, and the gynoid region was defined as the upper limit below the pelvis cut line by 1.5 times the height of the android region. The height of the gynoid region was equal to two times the height of the android region. All DXA scans were performed by the same trained professional and followed standard quality control procedures according to the manufacturer. The equipment was calibrated daily according to the manual's protocol (iDXA GE Medical Systems enCORE Operator's Manual and Product Information).

For the examination of waist circumference (WC) and hip measurement, in centimeters (cm) the participants were recommended to wear light clothing and without metal accessories, according to the standard protocol (Lohman et al. 1992). The waist-to-hip ratio (WHR) resulted from the division of the waist circumference by the hip measurement.

Height was measured with a wall stadiometer with an accuracy of 0.1 cm, while individuals remained barefoot in the orthostatic position and with their heads oriented in the horizontal position of the Frankfurt plane. The BMI calculation was the result of the total mass and height (kg/m^2).

Statistical analysis

The Hardy-Weinberg Equilibrium (HWE) tests, for the ancestry markers and SNPs, were performed using the Arlequin software version 3.5 (Excoffier & Lischer 2010).

Multiple linear regressions were used to examine the associations between each proportion of genetic ancestry and each genotype of the four SNPs with obesity-related measures in the sampled population. Since three female individuals do not have information on BMI and three other female individuals lack information on body fat percentage, and android and gynoid fat masses, the sample size varied in the analyses from 498 for these parameters to 501 for waist circumference and waist-to-hip ratio. Normality of continuous variables was tested by Shapiro Wilk test. All continuous parameters had a normal distribution.

Each ancestry — African, European and Amerindian — was used, through its proportions, as a continuous variable for the multiple linear regression analyses, and as nominal variables for the mean values assessment of the obesity-related traits in the sampled population. When used as nominal variable, AFR, EUR or NAM ancestry of the individuals from the population sample was defined according to the majority ancestry of each individual. To test the associations, six markers related to obesity were used: three anthropometric parameters namely BMI, waist circumference and waist-to-hip ratio, and three body composition measures such as body fat percentage, and android and gynoid fat masses. To avoid spurious associations, the following confounders were included in the analysis between ancestry and the obesity markers as control variables: age, gender and income.

For the analysis between SNP genotypes and the obesity traits, all the previous control variables were used, plus the continuous variable ancestry. Each genotype, TT, CT, CC for rs17782313 (*MC4R*); TT, AT, AA for rs9939609 (*FTO*); GG, AG, AA for rs7138803 (*FAIM2*); and CC, CT, TT for rs4074134 (*BDNF*), was encoded into a 'dummy variable' so that it was possible to use multiple linear regressions (Garavaglia & Sharma 1998, Draper & Smith 1998). The alleles described as having the highest risk associated with obesity and metabolic conditions are, in each SNP: C, A, A and C for rs17782313 (*MC4R*), rs9939609 (*FTO*), rs7138803 (*FAIM2*) and rs4074134 (*BDNF*),

respectively (Howe et al. 2021). The interpretation of the analyses' results with 'dummy variables' coding is in relation to the 'dummy variables' of reference, i.e., the heterozygous and homozygous genotypes of the risk alleles were analysed and interpreted in reference to the homozygous genotypes of lowest risk, which are not included in the association tests. Statistical analyses were performed using IBM SPSS software version 20.0 (IBM Corp., Armonk, NY, USA). A significance level of 0.05 was considered.

RESULTS

The descriptive characteristics of the population sample by the obesity-related markers are presented in Table I. The study consisted of 240 men and 261 women. The total average age of the sample was 51.62 years (SD = 7.90), and the total average income was 2246.05 BRL (SD = 1469.20). The average age for men and women was 51.23 years (SD = 7.81) and 51.68 years (SD = 7.98), respectively. The average income for men was 2260.49 BRL (SD = 1538.00) and for women was 2232.78 BRL (SD = 1405.84). Differences in mean values between men and women for the obesity-related traits revealed that variations were not statistically significant for BMI and android fat mass.

Analysis of population stratification

When individuals in a population do not breed randomly, the population is said to be stratified. In recently admixed populations, the presence of stratification implies the existence of groups with differences in their genetic composition.

Table I. Descriptive characteristics of the population sample containing means and standard deviations of total sample and between men and women for each obesity-related parameter.

	Total / Gender	n	Mean (SD)	*p* (between men and women)
BMI (kg/m²)	Total	498	28.04 (5.05)	0.635
	Men	240	27.93 (4.58)	
	Women	258	28.15 (5.46)	
WC (cm)	Total	501	97.10 (12.47)	0.003
	Men	240	98.81 (12.35)	
	Women	261	95.53 12.38)	
WHR	Total	501	0.94 (0.08)	0.001
	Men	240	0.97 (0.06)	
	Women	261	0.90 (0.07)	
BF (%)	Total	498	36.26 (8.32)	0.001
	Men	240	30.42 (6.40)	
	Women	258	41.69 (5.84)	
AFM (%)	Total	498	26.84 (11.82)	0.522
	Men	240	27.20 (12.49)	
	Women	258	26.51 (11.17)	
GFM (%)	Total	498	44.70 (18.64)	0.001
	Men	240	38.68 (14.44)	
	Women	258	56.10 (18.20)	

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist-to-Hip Ratio; BF: Body Fat; AFM: Android Fat Mass; GFM: Gynoid Fat Mass; N: number of individuals; SD: Standard deviation.

These differences must be taken into account in association studies, as they can lead to spurious association results. To check if the population is under stratification, HWE tests were carried out for all the genetic markers studied. The observed and expected heterozygosity values and corresponding *p*-values are detailed in Supplementary Material - Table SI.

Fourteen out of the 46 AIM-InDels showed statistically significantly lower heterozygozities than the expected ($p < 0.05$): MID-881, MID-3122, MID-593, MID-1644, MID-3854, MID-3072, MID-2264, MID-419, MID-493, MID-159, MID-2005, MID-1802, MID-1386 and MID-3626.

Among the four SNPs, rs4074134 (*BDNF*) had its observed heterozygosity (0.309) statistically significantly lower ($p = 0.001$) than the expected (0.395).

These results show deviations to Hardy-Weinberg Equilibrium, indicating population stratification. The homozygous charge is greater than the expected if it was a non-stratified population. In association studies, when a population is under stratification, spurious associations between genetic factors and characteristics linked to diseases may arise from differences in ancestry that are not related to the risk of disease itself.

The use of AIMs as a correction factor in association analyses between SNPs and the obesity-related markers related to a clinical condition is vital so that these stratification effects do not bias the results and lead to misinterpretations.

Genomic ancestry profile of the studied sample

A cluster analysis was performed, to estimate the proportions of African, European and Native American ancestry in the 501 individuals included in our sample, using the software STRUCTURE. This analysis was based on the 46 AIM-InDel profiles.

The average ancestry values in our sample from Rio de Janeiro were 28.80% African, 57.20% European, and 14% Native American. The results obtained reveal, however, a large variation among individuals (Figure 1). The variation in the proportions of Native American contribution was relatively small (SD = 0.11, Variance = 0.01) when

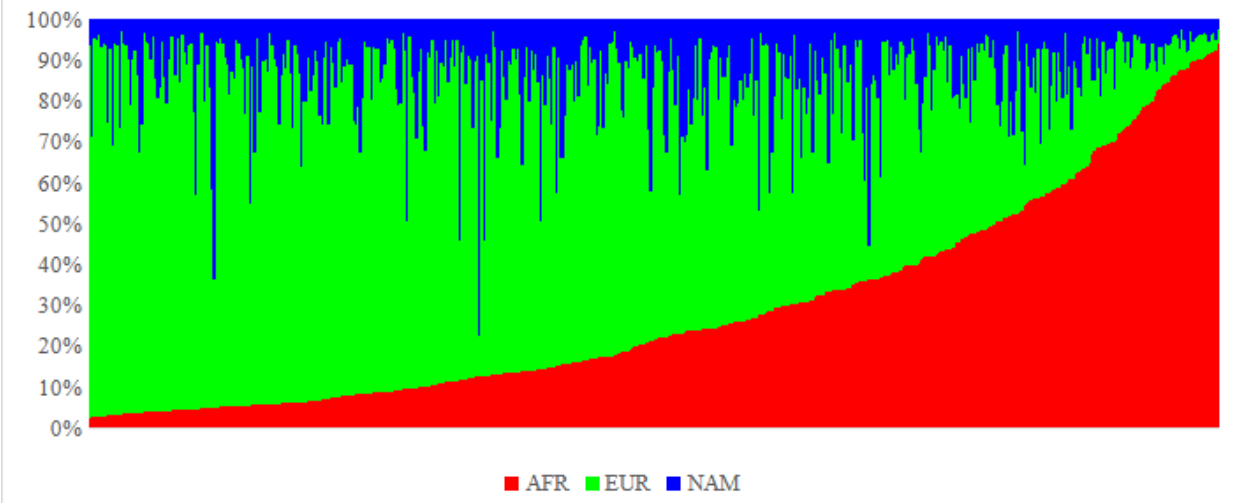

Figure 1. Genomic ancestry estimates of each individual in the population sample of the State of Rio de Janeiro. The average genomic ancestry values in the sample were 28.8% African, 57.2% European, and 14% Native American. Each column represents an individual and each color represents the corresponding relative ancestry proportion in relation to a reference population. The analyses were carried out using reference population (option USEPOPINFO in STRUCTURE), and considering K = 3.

compared to the African (SD = 0.25, Variance = 0.06) and European (SD = 0.25, Variance = 0.06) ancestries.

Obesity-related traits regarding the genomic ancestry of the sampled population

The quantitative characteristics of the obesity-related markers for each genetic ancestry of the population sample are summarized in Table II. Individuals with the majority of African ancestry exhibited the highest means for BMI, waist circumference, body fat percentage and gynoid fat mass, and the lowest for waist-to-hip ratio. The highest mean of android fat mass was detected in individuals with most European ancestry, while the highest for waist-to-hip ratio were observed in individuals with most European and most Native American ancestries alike. Individuals with most Native American ancestry showed the lowest means for all the obesity-related traits, with the exception of the waist-to-hip ratio, which there was no relevant difference between the three ancestries. There was no statistically significant difference observed.

Association between genomic ancestry and the obesity-related markers

Although we did not observe statistically significant associations, it is worth emphasizing the inverse association values between Native American ancestry and waist circumference, body fat percentage, android fat mass and gynoid fat mass (Table III). In the analysis without the inclusion of correction variables, according to the unstandardized regression coefficient (β), we found a statistically significant direct association between African ancestry and body fat percentage (β = 3.170, p = 0.036) and an inverse association between Native American ancestry and gynoid fat mass (β = -16.330, p = 0.040).

To avoid spurious interpretations, a new analysis was performed adjusting for age, gender and income variables. No statistically significant associations were detected. The new p-values were above the significance level between African ancestry and body fat percentage (β = -0.874, p = 0.459), and Native American ancestry and gynoid fat mass (β = -13.085, p = 0.065), which indicates an impact of the control variables in the unadjusted analysis.

It is worth noting that in our samples, as well as in a previously studied sample from the State of Rio de Janeiro population (Manta et al. 2013), most individuals have low Native American ancestry, and the variation in the African contribution is quite complementary to the European one. This may explain the opposite non-statistically significant associations observed between these two ancestries and the obesity-related markers.

Table II. Means, standard deviations and *p*-values of the obesity-related parameters for each genomic ancestry, as well as the frequencies of each ancestry in the population sample.

	AFR	EUR	NAM	*p*
n	**123**	**359**	**16**	
BMI (kg/m²)	28.54 (5.11)	27.97 (5.06)	25.81 (3.80)	0.111
WC (cm)*	97.69 (12.12)	97.09 (12.65)	92.81 (10.66)	0.338
WHR*	0.93 (0.07)	0.94 (0.08)	0.94 (0.08)	0.805
BF (%)	37.63 (8.78)	35.88 (8.17)	34.15 (6.74)	0.077
AFM (%)	26.92 (11.42)	27.03 (12.05)	22.06 (8.88)	0.257
GFM (%)	50.46 (19.19)	47.07 (18.60)	40.44 (11.71)	0.062

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist-to-Hip Ratio; BF: Body Fat; AFM: Android Fat Mass; GFM: Gynoid Fat Mass. *For WC and WHR n = 501: AFR = 125, EUR = 360, NAM = 16.

Table III. Multiple linear regression association and correlation analyses results, as well as confidence intervals and *p*-values, between the genomic ancestries and the obesity-related parameters.

	β (IC 95 %)	r	*p*
	No control variables		
BMI (kg/m²)			
AFR	1.051 (-0.756, 2.859)	0.051	0.254
EUR	-0.597 (-2.386, 1.192)	-0.029	0.512
NAM	-2.417 (-6.664, 1.829)	-0.050	0.264
WC (cm)			
AFR	-0.313 (-4.763, 4.137)	-0.006	0.890
EUR	1.468 (-2.930, 5.866)	0.029	0.512
NAM	-6.533 (-16.967, 3.900)	-0.055	0.219
WHR			
AFR	-0.018 (-0.045, 0.008)	-0.061	0.176
EUR	0.018 (-0.008, 0.045)	0.061	0.172
NAM	-0.002 (-0.065, 0.061)	-0.003	0.954
BF (%)			
AFR	3.170 (0.204, 6.137)	0.094	**0.036**
EUR	-1.881 (-4.817, 1.056)	-0.056	0.209
NAM	-6.813 (-13.772, 0.146)	-0.086	0.055
AFM (%)			
AFR	-0.680 (-4.913, 3.553)	-0.014	0.752
EUR	2.306 (-1.869, 6.480)	0.049	0.278
NAM	-9.246 (-19.137, 0.646)	-0.082	0.067
GFM (%)			
AFR	6.441 (-0.212, 13.095)	0.085	0.058
EUR	-3.383 (-9.968, 3.203)	-0.045	0.313
NAM	-16.330 (-31.920, -0.740)	-0.092	**0.040**
	With the control variables age, gender and income		
BMI (kg/m²)			
AFR	0.784 (-1.128, 2.696)	0.036	0.421
EUR	-0.264 (-2.135, 1.606)	-0.013	0.781
NAM	-2.518 (-6.786, 1.750)	-0.052	0.247
WC (cm)			
AFR	0.202 (-4.476, 4.881)	0.004	0.932
EUR	1.198 (-3.376, 5.772)	0.023	0.607
NAM	-7.237 (-17.664, 3.191)	-0.061	0.173
WHR			
AFR	-0.007 (-0.032, 0.017)	-0.027	0.553

Table III. Continuation.

EUR	0.009 (-0.015, 0.033)	0.033	0.469
NAM	-0.009 (-0.064, 0.046)	-0.015	0.746
BF (%)			
AFR	-0.874 (-3.191, 1.444)	-0.033	0.459
EUR	1.517 (-0.743, 3.777)	0.059	0.188
NAM	-3.560 (-8.721, 1.602)	-0.061	0.176
AFM (%)			
AFR	-0.428 (-4.924, 4.068)	-0.008	0.852
EUR	2.265 (-2.121, 6.651)	0.046	0.311
NAM	-9.670 (-19.662, 0.322)	-0.085	0.058
GFM (%)			
AFR	1.277 (-4.973, 7.527)	0.018	0.688
EUR	1.292 (-4.812, 7.395)	0.019	0.678
NAM	-13.085 (-26.979, 0.809)	-0.083	0.065

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist-to-Hip Ratio; BF: Body Fat; AFM: Android Fat Mass; GFM: Gynoid Fat Mass.

Association between the SNPs of MC4R, FTO, FAIM2, BDNF and the obesity-related markers

We observed a statistically significant direct association between the SNP 4074134 (*BDNF*) CC genotype and gynoid fat mass (Table IV).

In the analysis without correction variables, the unstandardized regression coefficient (β) denoted statistically significant inverse associations between the SNP rs7138803 (*FAIM2*) AG genotype, body fat percentage (β = -1.673, p = 0.032) and gynoid fat mass (β = -3.950, p = 0.024); and a statistically significant direct association between the SNP 4074134 (*BDNF*) CC genotype and gynoid fat mass (β = 6.049, p = 0.022).

A new analysis after adjusting for the variation in ancestry, age, sex and income was made and only the SNP 4074134 (*BDNF*) CC genotype direct association with gynoid fat mass persisted at the significance level (β = 6.232, p = 0.008), which points to an influence of the correction variables in the unadjusted association analysis between the SNP rs7138803 (*FAIM2*) AG genotype, body fat percentage (β = -0.014, p = 0.673) and gynoid fat mass (β = -0.051, p = 0.205). The SNP 4074134 (*BDNF*) CC genotype showed a significant higher percentage (6.23%) of gynoid fat mass compared to the lowest risk rs4074134 (*BDNF*) TT genotype.

Mean values of the obesity-related traits for each SNP genotype

Although there was no statistically significant difference noticed, we observed that individuals with the SNP highest risk genotypes have higher means of the obesity-related markers, with the exception of individuals with the highest risk genotypes of rs9939609 (*FTO*) for waist-to-hip ratio and rs7138803 (*FAIM2*) for body fat percentage and gynoid fat mass markers (Table V). This denotes, regardless of statistical significance, which depends on the sample size, some weight of the highest risk genotypes in the susceptibility of higher anthropometric and body composition measures, and consequently, adiposity.

Table IV. Multiple linear regression association and correlation analyses results, as well as confidence intervals and *p*-values, between the SNP genotypes and the obesity-related parameters.

	β (IC 95 %)	r	*p*
No control variables			
BMI (kg/m²)			
rs17782313 CT	0.536 (-0.424, 1.496)	0.043	0.273
rs17782313 CC*	1.013 (-1.090, 3.117)	0.035	0.344
rs9939609 AT	-0.542 (-1.542, 0.457)	-0.066	0.287
rs9939609 AA*	0.354 (-0.956, 1.664)	0.051	0.596
rs7138803 AG	-0.538 (-1.468, 0.392)	-0.056	0.256
rs7138803 AA*	0.249 (-1.480, 1.978)	0.026	0.777
rs4074134 CT	-0.118 (-1.625, 1.389)	-0.091	0.878
rs4074134 CC*	1.068 (-0.335, 2.472)	0.113	0.135
WC (cm)			
rs17782313 CT	1.106 (-1.256, 3.468)	0.034	0.358
rs17782313 CC*	2.678 (-2.511, 7.868)	0.039	0.311
rs9939609 AT	-0.587 (-3.048, 1.874)	-0.030	0.640
rs9939609 AA*	0.481 (-2.738, 3.700)	0.025	0.769
rs7138803 AG	-1.433 (-3.717, 0.852)	-0.064	0.218
rs7138803 AA*	1.371 (-2.890, 5.631)	0.043	0.528
rs4074134 CT	-0.299 (-4.023, 3.426)	-0.077	0.875
rs4074134 CC*	2.157 (-1.309, 5.623)	0.094	0.222
WHR			
rs17782313 CT	-0.007 (-0.021, 0.008)	-0.043	0.363
rs17782313 CC*	0.003 (-0.028, 0.034)	0.015	0.847
rs9939609 AT	-0.005 (-0.020, 0.010)	-0.024	0.516
rs9939609 AA*	-0.004 (-0.023, 0.016)	-0.004	0.704
rs7138803 AG	-0.003 (-0.017, 0.010)	-0.035	0.640
rs7138803 AA*	0.015 (-0.010, 0.041)	0.059	0.244
rs4074134 CT	0.002 (-0.021, 0.024)	0.040	0.886
rs4074134 CC*	0.010 (-0.011, 0.031)	0.057	0.353
BF (%)			
rs17782313 CT	1.405 (-0.169, 2.980)	0.070	0.080
rs17782313 CC*	2.321 (-1.134, 5.775)	0.047	0.187
rs9939609 AT	0.063 (-1.584, 1.710)	0.018	0.940
rs9939609 AA*	1.039 (-1.110, 3.188)	0.046	0.343
rs7138803 AG	-1.673 (-3.198, -0.147)	-0.093	**0.032**
rs7138803 AA*	-0.794 (-3.632, 2.044)	-0.001	0.583
rs4074134 CT	0.261 (-2.230, 2.752)	0.054	0.837
rs4074134 CC*	1.488 (-0.829, 3.805)	0.077	0.208

Table IV. Continuation.

AFM (%)			
rs17782313 CT	1.399 (-0.843, 3.641)	0.049	0.221
rs17782313 CC*	2.341 (-2.578, 7.260)	0.034	0.350
rs9939609 AT	-0.562 (-2.904, 1.779)	-0.032	0.637
rs9939609 AA*	0.562 (-2.493, 3.617)	0.029	0.718
rs7138803 AG	-1.388 (-3.562, 0.785)	-0.060	0.210
rs7138803 AA*	0.420 (-3.624, 4.463)	0.024	0.838
rs4074134 CT	0.415 (-3.110, 3.940)	0.085	0.817
rs4074134 CC*	3.130 (-0.150, 6.409)	0.119	0.061
GFM (%)			
rs17782313 CT	3.501 (-0.028, 7.029)	0.081	0.052
rs17782313 CC*	4.090 (-3.652, 11.832)	0.033	0.300
rs9939609 AT	0.237 (-3.450, 3.923)	0.027	0.900
rs9939609 AA*	3.565 (-1.245, 8.374)	0.070	0.146
rs7138803 AG	-3.950 (-7.367, -0.533)	-0.102	**0.024**
rs7138803 AA*	-0.795 (-7.152, 5.561)	-0.014	0.806
rs4074134 CT	1.847 (-3.708, 7.401)	0.078	0.514
rs4074134 CC*	6.049 (0.883, 11.216)	0.125	**0.022**
With the control variables age, gender and income; African, European and Native American ancestries			
BMI (kg/m²)			
rs17782313 CT	0.530 (-0.435, 1.495)	0.049	0.281
rs17782313 CC*	0.816 (-1.302, 2.934)	0.034	0.449
rs9939609 AT	-0.551 (-1.553, 0.451)	-0.049	0.280
rs9939609 AA*	0.392 (-0.931, 1.714)	0.026	0.561
rs7138803 AG	-0.054 (-1.537, 1.398)	-0.054	0.235
rs7138803 AA*	0.254 (-1.492, 2.000)	0.013	0.775
rs4074134 CT	-0.145 (-1.659, 1.370)	-0.009	0.851
rs4074134 CC*	1.018 (-0.400, 2.435)	0.064	0.159
WC (cm)			
rs17782313 CT	1.483 (-0.875, 3.841)	0.056	0.217
rs17782313 CC*	2.807 (-2.385, 8.000)	0.048	0.289
rs9939609 AT	-0.481 (-2.935, 1.973)	-0.017	0.700
rs9939609 AA*	0.963 (-2.269, 4.195)	0.026	0.558
rs7138803 AG	-0.080 (-1.356, 2.341)	-0.079	0.078
rs7138803 AA*	0.765 (-3.511, 5.042)	0.016	0.725
rs4074134 CT	-0.270 (-3.994, 3.453)	-0.006	0.887
rs4074134 CC*	2.149 (-1.333, 5.630)	0.055	0.226
WHR			
rs17782313 CT	0.001 (-0.012, 0.013)	0.003	0.940

Table IV. Continuation.

rs17782313 CC*	0.008 (-0.020, 0.036)	0.026	0.571
rs9939609 AT	-0.003 (-0.016, 0.010)	-0.019	0.678
rs9939609 AA*	0.003 (-0.014, 0.020)	0.016	0.724
rs7138803 AG	-0.081 (-0.097, 0.074)	-0.091	0.053
rs7138803 AA*	0.003 (-0.019, 0.026)	0.013	0.779
rs4074134 CT	0.003 (-0.017, 0.022)	0.012	0.792
rs4074134 CC*	0.009 (-0.010, 0.027)	0.042	0.347
BF (%)			
rs17782313 CT	0.570 (-0.598, 1.738)	0.043	0.338
rs17782313 CC*	0.809 (-1.759, 3.377)	0.028	0.536
rs9939609 AT	0.113 (-1.103, 1.328)	0.008	0.856
rs9939609 AA*	0.809 (-0.789, 2.406)	0.045	0.320
rs7138803 AG	-0.014 (-1.115, 1.258)	-0.019	0.673
rs7138803 AA*	0.343 (-1.775, 2.461)	0.014	0.750
rs4074134 CT	0.287 (-1.551, 2.124)	0.014	0.759
rs4074134 CC*	1.506 (-0.212, 3.224)	0.078	0.086
AFM (%)			
rs17782313 CT	1.536 (-0.724, 3.796)	0.060	0.182
rs17782313 CC*	2.400 (-2.568, 7.369)	0.043	0.343
rs9939609 AT	-0.540 (-2.895, 1.815)	-0.020	0.653
rs9939609 AA*	0.871 (-2.224, 3.966)	0.025	0.581
rs7138803 AG	-0.066 (-1.356, 2.457)	-0.065	0.148
rs7138803 AA*	0.226 (-3.869, 4.322)	0.005	0.914
rs4074134 CT	0.355 (-3.198, 3.908)	0.009	0.844
rs4074134 CC*	3.179 (-0.143, 6.500)	0.085	0.061
GFM (%)			
rs17782313 CT	1.938 (-1.208, 5.084)	0.055	0.227
rs17782313 CC*	1.764 (-5.152, 8.681)	0.023	0.616
rs9939609 AT	0.101 (-3.171, 3.373)	0.003	0.952
rs9939609 AA*	2.860 (-1.440, 7.160)	0.059	0.192
rs7138803 AG	-0.051 (-1.264, 3.021)	-0.057	0.205
rs7138803 AA*	1.456 (-4.242, 7.154)	0.023	0.616
rs4074134 CT	1.721 (-3.199, 6.642)	0.031	0.492
rs4074134 CC*	6.232 (1.632, 10.832)	0.120	0.008

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist-to-Hip Ratio; BF: Body Fat; AFM: Android Fat Mass; GFM: Gynoid Fat Mass. rs17782313 TT, rs9939609 TT, rs7138803 GG and rs4074134 TT are the lowest risk genotypes described in association with obesity and metabolic conditions (Ensembl Genome Browser 2021), and were used as reference for the other two genotypes in the analyses and interpretations. *Highest risk genotypes.

Table V. Means, standard deviations and *p*-values of the obesity-related parameters for each genotype of the four genetic loci, as well as the frequencies of each genotype in the population sample.

	n	BMI (kg/m²)	WC (cm)**	WHR**	BF (%)	AFM (%)	GFM (%)
rs17782313 TT	311	27.80 (5.20)	96.57 (12.67)	0.94 (0.08)	35.68 (8.46)	26.21 (11.81)	46.35 (19.47)
rs17782313 CT	163	28.36 (4.79)	97.72 (12.11)	0.93 (0.08)	37.09 (8.02)	27.67 (11.66)	49.86 (16.98)
rs17782313 CC*	24	**28.83 (4.67)**	**99.29 (12.16)**	**0.94 (0.07)**	**38.00 (8.32)**	**28.61 (12.73)**	**50.44 (18.06)**
rs9939609 TT	164	28.21 (5.32)	97.23 (13.26)	**0.94 (0.07)**	36.03 (8.32)	26.92 (12.70)	46.94 (17.91)
rs9939609 AT	246	27.71 (4.87)	96.72 (11.77)	0.93 (0.08)	36.10 (8.46)	26.45 (11.20)	47.19 (19.34)
rs9939609 AA*	88	**28.60 (5.02)**	**97.79 (12.89)**	0.93 (0.08)	**37.08 (8.00)**	**27.58 (11.80)**	**50.52 (18.07)**
rs7138803 GG	251	28.22 (5.18)	97.55 (12.70	0.93 (0.08)	**37.02 (8.27)**	27.33 (11.83)	**49.44 (18.15)**
rs7138803 AG	209	27.71 (4.78)	96.17 (12.14)	0.93 (0.07)	35.35 (8.44)	26.01 (11.47)	45.48 (17.22)
rs7138803 AA*	38	**28.50 (5.61)**	**98.97 (12.63)**	**0.95 (0.07)**	36.23 (7.68)	**27.82 (13.41)**	48.64 (27.12)
rs4074134 TT	59	27.34 (4.78)	95.71 (11.87)	0.93 (0.07)	35.28 (9.38)	24.60 (11.26)	43.58 (19.14)
rs4074134 CT	152	27.35 (4.57)	95.65 (11.76)	0.93 (0.08)	35.58 (8.39)	25.33 (10.80)	45.51 (16.22)
rs4074134 CC*	287	**28.53 (5.30)**	**98.11 (12.87)**	**0.94 (0.07)**	**36.81 (8.04)**	**28.04 (12.30)**	**49.71 (19.53)**
p		0.511	0.193	0.092	0.374	0.453	0.492

BMI: Body Mass Index; WC: Waist Circumference; WHR: Waist-to-Hip Ratio; BF: Body Fat; AFM: Android Fat Mass; GFM: Gynoid Fat Mass; SD: Standard Deviation. *Highest risk genotypes. **For WC and WHR n = 501: rs17782313 TT = 313, rs17782313 CT = 164, rs17782313 CC = 24; rs9939609 TT = 166, rs9939609 AT = 246, rs9939609 AA = 89; rs7138803 GG = 252, rs7138803 AG = 211, rs7138803 AA = 38; rs4074134 TT = 59, rs4074134 CT = 152, rs4074134 CC = 290.

DISCUSSION

Ancestry informative markers

The European and African demographic impact in Brazil is historically well documented and supported by genetic studies. Uniparental markers showed clear evidence of a biased admixture involving predominantly descendants of European men and African women (Alves-Silva et al. 2000, Carvalho-Silva et al. 2001, Hünemeier et al. 2007).

Studies using autosomal AIMs have demonstrated an essentially tri-hybrid heritage and a predominantly European ancestry in most Brazilian populations, with differences in ancestry proportions depending on the geographic region considered or in different self-declared ethnic groups (de Souza et al. 2019, Pena et al. 2020).

The results from this work are also in line with those from Manta et al. (2013). Through the analysis of 46 AIM-InDels in a population sample from Rio de Janeiro, they found a predominantly European ancestry (55.20%), followed by African (31.10%) and Amerindian (13.70%).

Considering the results described above, it is possible to conclude that the population ancestry estimates obtained are in accordance with previous studies, as well as with historical data concerning the origin of the Brazilian population.

Obesity-related traits

The obesity-related measures used in this study are good indicators of nutritional status and predictors of overweight and obesity. The BMI, waist circumference, waist-to-hip ratio and body fat percentage are the most

used parameters in epidemiological studies. The present study has both strengths and limitations. Some of its strengths are its study design, ancestry estimation and anthropometric and body composition assessment. The latter includes the evaluation of body fat, android and gynoid fat masses as measured via DXA, therefore overcoming the limitations of previous studies that used only reported weights and anthropometric measurements such as BMI, WC, and WHR. The DXA accurately reflects the measures of body composition. This method allows accurate quantifications of total body fat as well as its distribution and location. The DXA is considered the gold standard in validation studies of methods and equations, and it is noninvasive, precise, and automatic (da Rocha et al. 2017).

Some study limitations should be mentioned. The sample size and the absence of information on physical activity, dietary habits and other possible residual confounding, which are recognized to influence our main outcome variables, may have limited our conclusions. Being a cross-sectional study, another limitation is the inability to establish temporality.

Genomic ancestry and the obesity-related traits

In general, studies examining associations between African or European ancestry using AIMs and markers related to obesity such as BMI report uniform findings. Direct associations are observed between African ancestry and traits such as BMI and waist circumference, while inverse relationships are detected with European ancestry. Inverse associations were also observed with Native American ancestry. However, the authors emphasize the important role of the environment and socioeconomic components in these results (Fernández et al. 2003, Tang et al. 2006, Lai et al. 2009, Cheng et al. 2010, Lins et al. 2012, Nassir et al. 2012, Goonesekera et al. 2015, Klimentidis et al. 2016).

In the study by Nassir et al. (2012) in post-menopausal African-American and Hispanic-American participants of the Women's Health Initiative (WHI), significant direct associations were found between African ancestry and BMI in the general population, as well as in subgroups of African-Americans and Hispanic-Americans. However, when waist-to-hip ratio was used as a parameter of obesity, a significantly weaker direct association with African ancestry was found in the general population and unobserved among African-Americans or Hispanic-Americans. Interestingly, Amerindian ancestry was significantly direct associated with waist-to-hip ratio, but not with BMI in the general population.

Amerindian ancestry showed non-significant inverse associations for all obesity-related markers used in this study. One explanation may be the diet and healthy lifestyle. Goonesekera et al. (2015) observed evidence of a modifying effect on the associations between Native American ancestry and adiposity by diet. Individuals with this ancestry who had a relatively high healthy eating score, had inverse associations with all anthropometric and body composition measures, although not all results have reached statistical significance.

We did not replicate the previous findings of a statistically significant association between genomic ancestry, anthropometric parameters and body composition measures. The differences in environmental, social, economic and cultural conditions between the populations investigated in the different studies may explain our lack of significant association. However, our findings must be further replicated in larger samples using populations from the same origin.

When studying characteristics related to health, biodiversity and variability attributed

to the use of genetic admixture estimates, they should not be simply replaced by the simple classification of ethnicity, but used together. Self-declaration of ethnicity is easier to access and important to personal identification, but its exclusive use confuses the historical ancestry of individuals and the understanding of the biological factors underlying obesity, its traits related to metabolic risks, and its variation within and between populations (Fernández et al. 2003).

As populations continue to breed, self-identification tends to become less a reflection of an individual true ancestral origin. Divers et al. (2011) described this trend in which, among Hispanic Americans in the Multi-Ethnic Study for Atherosclerosis (MESA), genomic ancestry did not correspond to Hispanic self-declaration in 30% of its self-declared Hispanic participants.

Relationship between the SNPs and the obesity-related traits

rs17782313 (MC4R)

A possible explanation for not finding a statistically significant association between this locus and obesity-related markers, in this study, is that it may be more associated with obesity in childhood than in adulthood (Hardy et al. 2009). The rs17782313 showed stronger direct associations with total mass than with BMI. Its association with mass increased during childhood and adolescence, peaked at the age of 20, and weakened during adulthood. It was concluded that the genetic variants in *MC4R* showed similar biphasic changes in their associations with BMI and weight, respectively, strengthening during childhood until the age of 20 and weakening with increasing adulthood.

We failed to identify a significant association between the SNP and the obesity-related measures in a Brazilian population sample. Da Fonseca et al. (2019) observed a similar result. They found no significant association between the SNP and obesity in a cohort of 490 individuals from Brazil. Grant et al. (2009) also found a similar result, in which no significant association was observed between rs17782313 (*MC4R*) and obesity in Afro-American individuals. These findings suggest that the influence of this polymorphism on the obesity risk may also be dependent on the ethnic group, and on different environmental, social and cultural characteristics.

rs9939609 (FTO)

In a recent study in which male carriers of the risk allele (A) were investigated, they had body mass, BMI, WHR and waist and hip circumferences significantly higher than men without this risk allele. They were also more frequently diagnosed with obesity based on BMI and waist circumference. This influence was not seen in women. The results point to a male-specific association between the *FTO* polymorphism and obesity characteristics. (Zdrojowy-Wełna et al. 2020). Manco et al. (2019), in turn, observed an direct association between rs9939609 (*FTO*) and obesity or body fat indices in female adolescents, but not in males.

The role of *FTO* variants on obesity risk is consistent in studies with European ancestry individuals (Dina et al. 2007). However, apparently it is not associated with obesity risk in African Americans, Chinese Han, and Native Oceanic populations (Ohashi et al. 2007, Scuteri et al. 2007, Li et al. 2008).

In our investigation, we did not replicate the previous findings that suggest significant association between the polymorphism and the obesity-related parameters. Other studies have analysed the relationship between rs9939609 (*FTO*) and obesity susceptibility in Brazil. Reuter et al. (2016) studied the association

between this SNP and overweight/obesity risk in a sample of youths from the South of Brazil. They observed that the rs9939609 (*FTO*) was significantly associated with increased BMI and waist circumference. Pereira et al. (2016) have performed a similar study with children. However, they did not find this association.

Ramos et al. (2012) investigated the contribution of rs9939609 (*FTO*) in morbid obesity risk. Their results suggested that the presence of the rs9939609 polymorphism risk allele increased the risk for obesity. More recently, Da Fonseca et al. (2019, 2020) observed that this polymorphism plays a significant role in predisposing higher body weight, BMI and severe obesity in a Brazilian population sample. The conflicting results may be explained by the differences in social and cultural conditions of the individuals, such as income and eating habits.

rs7138803 (FAIM2)

Our study is the first in a Brazilian population to investigate the association of rs7138803 (*FAIM2*) with obesity-related adiposity markers. Although we did not observe statistically significant association in our population sample, other authors show an association of this polymorphism with obesity-related parameters, mainly in European and Asian populations. These contrasting findings are probably due to significant differences in ethnic, environmental, social and cultural components along the continents.

Thorleifsson et al. (2009) found that rs7138803 (*FAIM2*) was associated with increased BMI and obesity based on studies of GWAS in individuals of European ancestry. Subsequently, a replicated study showed that this SNP was significantly associated with higher BMI, waist circumference and obesity risk in the Chinese population (Wu et al. 2010).

Recently, in Chinese Han population, Kang et al. (2020) observed associations with increased BMI, diastolic blood pressure and triglycerides. However, in the same Han population, Li et al. (2013) detected no significant effect of rs7138803 (*FAIM2*) in obesity. Another study showed that the polymorphism was associated in adiposity regulation in both Chinese Han individuals and Europeans (Ng et al. 2010). In Europeans, the rs7138803 (*FAIM2*) seems to contribute to variation in body morphology and composition in Spanish people. (Poveda et al. 2014).

rs4074134 (BDNF)

Xu et al. (2003) observed that, similar to the *MC4R* mutations, mice with mutations that express decreased amounts of the *BDNF* receptor showed hyperphagia and obesity at the beginning of maturity, suggesting a role of the gene in the energy balance.

In the present study, we observed a significant association between the rs4074134 (*BDNF*) and gynoid fat mass. Gynoid fat accumulation is often associated with a protective effect on cardiovascular risk, as opposed to android fat. Accumulation in gynoid fat is associated with a decreased risk of type 2 diabetes mellitus and cardiovascular risk, which in part may reflect the downregulation of proinflammatory signaling pathways and insulin resistance. The mechanisms for the cardiometabolic benefits of gynoid fat relate to its inverse association with insulin resistance and include less expression of proinflammatory cytokines compared with android fat mass (Min & Min 2015, Miller et al. 2020).

In contrast, Wiklund et al. (2008) observed that gynoid fat mass was significantly associated with impaired glucose tolerance, hypertriglyceridemia and hypertension and consequently cardiovascular risk, mainly in

men. The inconsistency of the results may rely on possible confounding factors not adjusted.

Since most of the studies investigating the relationship between rs4074134 (*BDNF*) and obesity have been carried out in Asian populations, there is still much to be clarified in populations from other continents. Assessing the influence of fat distribution, and gynoid fat mass in particular, on cardiovascular risk endpoints such as stroke and heart diseases requires further investigation.

CONCLUSIONS

Our results suggest that the rs4074134 (*BDNF*) CC genotype was directly associated with gynoid fat mass in the population sample, independently of age, gender, income and genomic ancestry of the individuals. Statistically significant association was restricted to gynoid fat mass evaluated via DXA, with no evidence of association with the other body composition measures neither with the measured anthropometric indices. Given the high validity of our outcome measurements, this study findings strengthen available evidence about genetic factors on fat accumulation. We did not observe statistically significant associations between genomic ancestry and the markers related to obesity. We highlight, although not statistically significant, the results values of inverse association between Native American ancestry and the obesity-related traits in the sampled population. We strongly recommend association studies between genetic factors and obesity-related measures with larger sample sizes in the Brazilian population.

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SUPPLEMENTARY MATERIAL

Table SI.

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ALLAN SCHARF[1]
https://orcid.org/0000-0001-7579-5598

FLÁVIA F. BEZERRA[2]
https://orcid.org/0000-0002-6594-4323

VERÔNICA M. ZEMBRZUSKI[3]
https://orcid.org/0000-0002-0044-3210

ANA CAROLINA P. DA FONSECA[3,4]
https://orcid.org/0000-0001-6123-0481

LEONOR GUSMÃO[1]
https://orcid.org/0000-0003-0432-6481

EDUARDO FAERSTEIN[5]
https://orcid.org/0000-0002-4027-4896

[1]State University of Rio de Janeiro (UERJ), DNA Diagnostic Laboratory, São Francisco Xavier Street, 524, 20550-900 Rio de Janeiro, RJ, Brazil

[2]State University of Rio de Janeiro (UERJ), Institute of Nutrition, São Francisco Xavier Street, 524, 20550-900 Rio de Janeiro, RJ, Brazil

[3]Oswaldo Cruz Foundation (FIOCRUZ), Human Genetics Laboratory, Oswaldo Cruz Institute, Brasil Avenue, 4365, 21040-360 Rio de Janeiro, RJ, Brazil

[4]Oswaldo Cruz Foundation (FIOCRUZ), Laboratory of Immunopharmacology, Oswaldo Cruz Institute, Brasil Avenue, 4365, 21040-360 Rio de Janeiro, RJ, Brazil

[5]State University of Rio de Janeiro (UERJ), Institute of Social Medicine, São Francisco Xavier Street, 524, 20550-900 Rio de Janeiro, RJ, Brazil

Correspondence to: **Allan Scharf**
E-mail: allanscharf@hotmail.com

Author contributions

Allan Scharf, Flávia Fioruci Bezerra, Leonor Gusmão and Eduardo Faerstein contributed to the study conception and design. Material preparation, data collection, and analyses were performed by Allan Scharf. Verônica Marques Zembrzuski and Ana Carolina Proença da Fonseca made the SNPs genotyping. Allan Scharf wrote the manuscript, and all authors commented and read the previous versions of the document and approved the final manuscript.