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# *FTO* and *NOS3* genes associated with pediatric obesity: Corações de Ouro Preto study

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## Abstract

**Background** Obesity is the largest global public health epidemic, increasingly affecting children and adolescents. Studies suggest that genetic markers such as single nucleotide polymorphisms (SNPs) may be associated with the development of obesity. Obesity susceptibility genes identified include alpha-ketoglutarate-dependent dioxygenase (*FTO*), endothelial nitric oxide (*NOS3*) and apolipoprotein B (*APOB*). Furthermore genetic predisposition can interact with other environmental factors, such as clinical risk factors for obesity. In this context, the potential interaction between these SNPs and clinical risk factors such as non-exclusive breastfeeding, high birth weight, and a family history of chronic diseases warrants investigation. There is a clear need for more research on the *FTO*, *NOS3* and *APOB* genes in Brazilian children. The purpose of this study was to evaluate the associations between SNPs in the *FTO* (rs1121980), *NOS3* (rs1799983) and *APOB* (rs693) genes and obesity as well as to investigate the combined influence of significant SNPs in children and adolescents in Ouro Preto, Minas Gerais, Brazil.

**Methods** A cross-sectional population-based study was conducted with elementary school students aged 6–17 years in Ouro Preto, Minas Gerais, between April and December 2021. The study evaluated sociodemographic, clinical, and biochemical variables and the SNPs rs1121980, rs1799983 and rs693 in the *FTO*, *NOS3 and APOB* genes, respectively, for associations with obesity.

**Results** The study revealed that the prevalence of obesity was notably high, reaching 8.5% in the study population. Homozygotes for the risk alleles of the *FTO* and *NOS3* genes (genotypes AA and TT, respectively) remained significant, with both showing a more than twofold increased likelihood of being obese [OR: 2.07 (CI: 1.02–4.20) and 2.49 (CI: 1.08–5.73), respectively]. The same combination of alleles associated with clinical risk factors (nonexclusive breastfeeding, high birth weight, family history of diabetes, obesity and dyslipidemia) was associated with a significantly greater chance of being obese at a young age.

**Conclusions** Our results support the idea that the SNP rs1121980 in the *FTO* gene and rs1799983 in the *NOS3* gene can affect the occurrence of obesity in Brazilian children and adolescents living in urban areas.

**Keywords** Obesity, Adolescent health, Native American child health, Genetics, Epidemiology

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## Background

Obesity, a multifactorial and polygenic condition, is the largest global public health epidemic and increasingly affects children and adolescents [1]. Body adiposity is hereditary, making the identification of genes that contribute to the most common forms of obesity critically important. The alpha-ketoglutarate-dependent dioxygenase (*FTO*) gene was identified as the first obesity susceptibility gene [2, 3]. The presence of single nucleotide polymorphisms (SNPs) clustered in the first intron of the *FTO* gene has been associated with an increased risk of obesity in adults [3]. One SNP located in this region is rs1121980, where a guanine (G) is replaced by an adenine (A). The frequency of the A allele is around 53% in obese people compared to 40.7% in eutrophic people [3]. In addition, the HapMap project shows that the global distribution of the A allele is 41% and that of the G allele is 59%. For Americans, this distribution is approximately 36% and 64%, respectively [4]. Studies indicate that this SNP affects the transcriptional activity of the gene and is associated with increased body mass index (BMI), waist circumference (WC), and extreme obesity in individuals carrying the variant allele A [2, 5, 6]. The endothelial nitric oxide (*NOS3*) gene is also under investigation for its association with obesity [7]. The SNP rs1799983 is located in exon 7 of the *NOS3* gene, where a G is substituted by a thymine (T), resulting in the substitution of a glutamate for an aspartate at position 298 of NOS3 (Glu298Asp), which compromises its functionality [8]. According to the HapMap project, the worldwide distribution of the T allele is 17% and that of the G allele is 83%, while in Americans this distribution increases to 34% and 66%, respectively [9]. Studies suggest that the SNP rs1799983 is associated with the development of obesity, systemic hypertension, coronary artery disease, alterations in low-density lipoprotein (LDL-c) and high-density lipoprotein (HDL-c) fractions, and diabetes mellitus type 2 (DM2) [10–14].

The SNP rs693 or XbaI is located in exon 26 of the *APOB* gene, where there is a G to A substitution. The presence of the A allele has been extensively studied for its involvement in generalised or regional obesity and dyslipidemia [15–18]. Similarly, studies in Brazilian populations show the same results [19, 20]. The global frequency of the A risk allele is 23%. In the American population it is about 29% [21].

Furthermore, genetic predispositions can interact with other environmental factors, such as clinical risk factors for obesity. In this context, the potential interaction between the *FTO*, *NOS3 and APOB* SNPs and clinical risk factors such as nonexclusive breastfeeding, high birth weight, and a family history of chronic diseases warrants investigation. These clinical factors are well-established contributors to obesity [22], and their combination with genetic risk may synergistically increase the likelihood of obesity development in children and adolescents. Studies conducted in Ouro Preto have examined both adult and child populations, revealing a high prevalence of overweight and obesity [23–25]. There is a clear need for more research on the *FTO* and *NOS3* genes in Brazilian children. The purpose of this study was to evaluate the associations between SNPs in the *FTO* (rs1121980), *NOS3* (rs1799983) and *APOB* (rs693) genes and obesity as well as to investigate the combined influence of significant SNPs in children and adolescents in Ouro Preto, Minas Gerais, Brazil.

## Methods

### Study design and population

A population-based cross-sectional study was performed with elementary school students aged 6–17 years in Ouro Preto, Minas Gerais, between April and December 2021.

### Sampling plan

A stratified sample size for the population survey was calculated on the basis of four parameters: (1) 14.9% of the population in the studied age group with overweight and obesity; (2) 4,864 students enrolled in public and private elementary schools in Ouro Preto; (3) a 3% margin of error; and (4) a 95% confidence level. This resulted in a minimum sample size of 876, which was increased by 20% to account for potential losses, leading to the invited of 1,168 individuals. An 18.8% loss rate due to nonattendance and low blood volume resulted in a final sample of 921 individuals.

### Inclusion and exclusion criteria

The inclusion criteria were as follows: the student had to be enrolled in primary school in Ouro Preto, and the person responsible for the student to be interviewed had to be over eighteen years old and have lived in the same household as the student. The exclusion criteria were pregnant adolescents, students in youth and adult education, and the Association of Parents and Friends of the Exceptional.

### Data collection

Data collection consisted of the following steps: (1) a face-to-face interview with the student's legal guardians, who answered a questionnaire about the student's sociodemographic, behavioral, and clinical data; (2) a physical examination consisting of weight and height measurements; and (3) blood tests for biochemical and molecular evaluation of SNPs.

### Questionnaire

#### *Sociodemographic*

Sociodemographic data included data such as gender, age, skin color (self-reported), school (private or public), family income (minimum wage) and the schooling of the chief of the family. In 2021, the minimum wage was approximately 207 dollars.

#### *Clinical*

Neonatal history (gestational age and birth weight), breastfeeding and family history of obesity, DM2 or dyslipidemia were recorded.

### Anthropometric parameters

#### *Weight*

Weight was measured on a Tanita Ironman InnerScan® digital anthropometric scale in an orthostatic position wearing light clothing.

#### *Height*

Height was measured with a Sanny® field stadiometer, graduated in millimeters (mm), with the student standing with his back to the marker, with bare feet together, in an orthostatic position, and looking straight ahead.

#### *BMI*

BMI was calculated by weight (in kilogram) divided by height squared (in meters), being classified as eutrophic (if BMI/age ≤ + 1 in the Z score) or excess weight (if BMI/age > + 1 in the Z score) [26].

### Biochemical analysis

For the biochemical analysis, the participant was in a stable metabolic state with the usual diet state, and as recommended for the child population, fasting was not needed [27, 28].

#### *Glycated hemoglobin*

Following the manufacturer's protocol, the glycated hemoglobin (HbA1c) level was measured via immunoturbidimetry on a Cobas Integra 400 plus® automatic analyzer (Roche, Germany). The HbA1c level was classified as normal (if < 5.7%) or high (if ≥ 5.7%) [29].

#### *Lipid profile*

The serum concentrations of triglycerides (TG), total cholesterol (TC), and HDL-c were measured via the enzymatic–colorimetric method (Triglicérides Monoreagent®, Cholesterol Monoreagent®, HDL Direto®, Bioclin/Quibasa, Brazil) and evaluated via Chemwell R6® Automated Analyzer, Awareness Technology. The Friedewald formula was used to calculate the LDL-c fraction: LDL-c (mg/dL) = (TC-HDL-c) - (TG/5), applicable when the concentration of TG is ≤ 400 mg/dL [30]. The Brazilian Society of Cardiology [31] criteria were used to classify dyslipidemia. According to this criteria, a normal value is TC < 170 mg/dL, LDL-c < 110 mg/dL, HDL-c >45 mg/dL, and TG < 85 mg/dL (for ages 0 to 9) and < 100 mg/dL (for ages 10 to 19).

#### *Molecular analysis*

Genomic DNA extraction was performed via the Wizard® Genomic DNA Purification Kit (Promega®) according to the manufacturer's protocol. The SNPs rs1121980, rs1799983 and rs693 were chosen on the basis of the following criteria: (1) positive association with obesity in previous studies, including GWAS, in similar ethnic groups as the study population [2, 3, 17, 32, 33]; and (2) absence of rare alleles in studies with African and European populations. The allele and genotype frequencies were evaluated and tested for Hardy–Weinberg equilibrium (HWE). The allelic discrimination technique was performed via real-time PCR and a set of primers and probes specific for each SNP (TaqMan® Minor Groove Binder-MGB, TaqMan® System; 7500 fast real-time PCR Systems, Applied Biosystems). The next steps involved initial denaturation at 95 °C for 10 min, 40 cycles of annealing at 95 °C for 15 s, and a final extension at 60 °C for 1 min. Two negative controls were included for quality control and to detect potential contamination. The results were read, and samples underwent supplementary amplification if there was uncertainty in interpretation.

### Exposure, outcome, and confounders

Sociodemographic, clinical, anthropometric, and biochemical variables and the rs1121980, rs693 and rs1799983 SNPs were evaluated as exposures. Obesity was considered the outcome.

#### *Genetic score*

Considering the SNPs that were statistically significant in the bivariate analysis, a genetic score was constructed to assess the combined influence of rs1121980 and rs1799983 on the association with obesity. Although rs693 was initially analyzed, it was not included in the genetic score as it did not show statistical significance in the univariate analysis. This score, which is based on the frequency of alleles considered "risk" or "protective," was defined according to the literature. The criteria were as follows: (1) rs1121980, characterized by the exchange of G for A, where allele G (wild type) is considered protective and allele A (altered) is a risk allele. (2) rs1799983 is characterized by the exchange of G for T, where allele G (wild type) is considered protective and allele T (altered) is a risk allele. (3) The total score for everyone was obtained by summing the genotypes of the SNPs as follows: Σ = *FTO* genotype + *NOS3* genotype. The scoring system allocated 0 points for the protective allele, 1

point for one risk allele, and 2 points for two risk alleles. Thus, the total genetic risk score, derived from the points of both SNPs, could range from 0 to 4. A score of 0 indicated that the individual carried protective alleles for both SNPs, whereas a score of 4 indicated the presence of two risk alleles in both SNPs.

### Statistical analysis

The database was built and analyzed via Stata software version 15.0. The participants were characterized through bivariate analysis. Pearson's chi-square test or Fisher's exact test was used for differences in these groups. The allele frequencies were obtained via gene counting and tested for HWE.

Logistic regression was conducted to assess the link between SNPs and obesity. A theoretical causality model using a directed acyclic graph (DAG) was created to guide the analysis (Dagitty® version 3.2). The DAG outlined the exposure variable (SNPs), outcome (obesity), and covariates (Fig. 1). To avoid unnecessary adjustments, spurious associations, and estimation errors, the backdoor criterion was employed to identify a minimal set of confounding variables [34]. Two models were proposed on the basis of the DAG: (1) adjusting for skin color alone to eliminate backdoor biases and (2) adjusting for age, gestational weight, skin color, family income, glycemic and lipid profile. Both models were analyzed to compare their differences. The variance inflation factor was used to assess collinearity among covariates via the "subsetByVIF" package with a maximum cutoff of 10 (VIF < 10) [35].

In addition to the primary analyses conducted on the total sample, we performed a stratified analysis by age, categorizing participants into two groups: children (6–9 years) and adolescents (10–17 years). This approach aimed to assess potential differences in the association between the studied polymorphisms and obesity across age groups. The results of these stratified analyses are available in the Supplementary Material.

An additive interaction analysis was performed to assess whether the impact of the number of risk alleles on the likelihood of obesity was influenced by other clinical factors. In the models, the number of risk alleles was treated as a continuous variable, whereas the other factors were included as categorical predictors. To explore potential modification effects, we included a first-degree (additive) interaction term between the number of risk alleles and each categorical variable in the models via the interaction operator #. This allowed us to evaluate whether the impact of risk alleles on obesity varied with different levels of the explanatory variables. By incorporating this interaction term, the model estimated the differential effect of risk alleles on the likelihood of obesity for each category of clinical risk factor, helping us identify and quantify how the combination of genetic risk factors and clinical conditions influences obesity development.

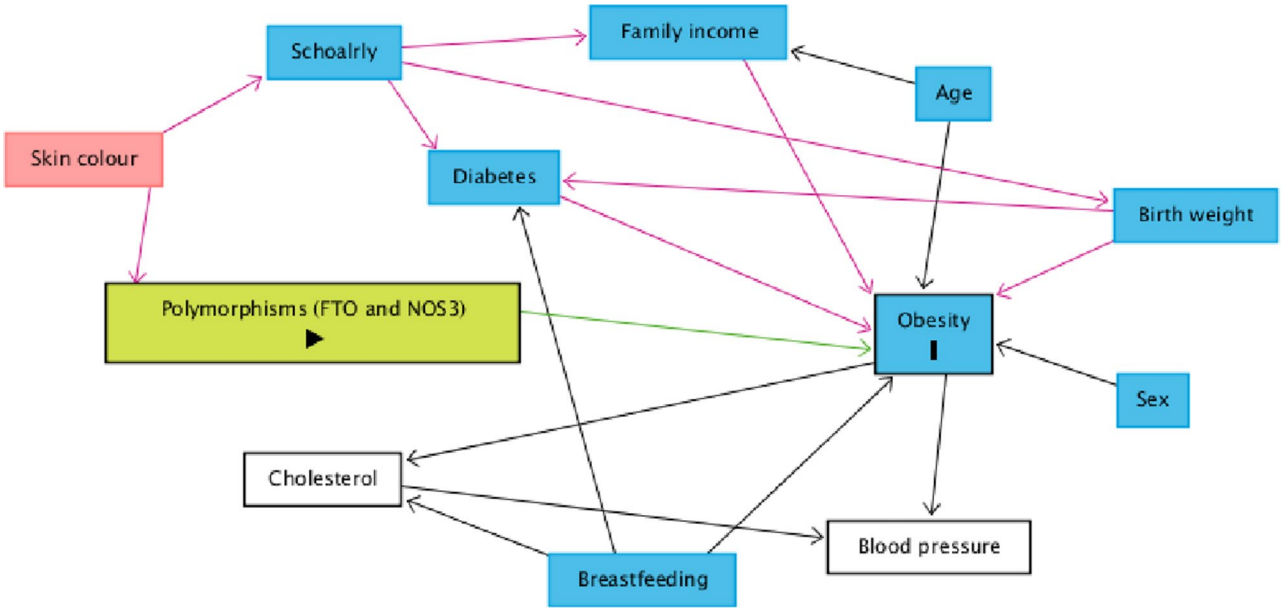


**Fig. 1** DAGs on the influence of SNPs in the *FTO* and *NOS3* genes on obesity. Description: Causal connections represented by arrows were established between variables. The variable in green with the symbol "►" is the exposure variable; the variable in blue with the letter "I" is the outcome variable; the blue variables are antecedents of the outcome variable; the pink variables are antecedents of the outcome and exposure variables; and the white variable is a collider and indicates a closed or blocked path

## Results

The study involved 921 students, 43.0% of whom were children (6–9 years) and 57.0% of whom were adolescents (10–17 years). For children and adolescents, the median ages were 7 (IQR: 6–8) and 12 (IQR: 11–14) years, respectively. The prevalence of obesity is 8.5%. Table 1 presents the sociodemographic and clinical characteristics of the individuals.

Significant differences in biochemical variables (HbA1c, HDL-c, and TG) and the AA genotype at rs1121980 and the TT genotype at rs1799983 were detected between students with and without obesity (Table 2). rs1121980 and rs1799983 were consistent with HWE ($p = 0.466$ and $p = 0.899$, respectively). rs693 showed no difference between groups. It was therefore removed from the adjusted analyses.

Table 3 presents the associations between the SNPs rs1121980 and rs1799983 and obesity based on two theoretical models suggested by the DAG (Fig. 1). After the necessary adjustments, homozygotes for the risk alleles of rs1121980 and rs1799983 (genotypes AA and TT,

**Table 1** Sociodemographic, behavioral, and clinical characteristics related to obesity in children and adolescents

| Characteristics | Total, *N* (%) | Non obese, *N* (%) | Obese, *N* (%) | Odds ratio, (CI95%) | *p*-value [a] |
|---|---|---|---|---|---|
| **Sociodemographic variables** | | | | | |
| **Sex** | | | | | |
| Female | 452 (49.1) | 420 (49.7) | 32 (42.1) | 1.00 | |
| Male | 469 (50.9) | 425 (50.3) | 44 (57.9) | 1.36 (0.84–2.18) | 0.206 |
| **Age (years)** | | | | | |
| 6–9 | 393 (42.7) | 356 (42.1) | 37 (48.7) | 1.00 | |
| 10–17 | 528 (57.3) | 489 (57.9) | 39 (51.3) | 0.77 (0.48–1.23) | 0.270 |
| **Skin color** | | | | | |
| White | 288 (31.3) | 270 (31.9) | 18 (23.7) | 1.00 | |
| Brown | 426 (46.2) | 387 (45.8) | 39 (51.3) | 1.51 (0.85–2.70) | 0.162 |
| Black | 207 (22.5) | 188 (22.3) | 19 (25.0) | 1.52 (0.77–2.97) | 0.224 |
| **School** | | | | | |
| Private | 124 (13.5) | 118 (14.0) | 6 (7.9) | 1.00 | |
| Public | 797 (86.5) | 727 (86.0) | 70 (92.1) | 1.89 (0.80–4.46) | 0.144 |
| **Family income** | | | | | |
| > 10 MW | 40 (4.3) | 39 (4.6) | 1 (1.2) | 1.00 | |
| 4–10 MW | 146 (15.7) | 136 (16.0) | 10 (12.3) | 2.87 (0.36–23.10) | 0.322 |
| 2–4 MW | 250 (26.9) | 222 (26.2) | 28 (34.6) | 4.92 (0.65–37.21) | 0.123 |
| ≤ 2 MW | 493 (53.1) | 451 (53.2) | 42 (51.9) | 3.63 (0.49–27.11) | 0.209 |
| **Schooling of the chief of family** | | | | | |
| High | 194 (21.1) | 181 (21.4) | 13 (17.1) | 1.00 | |
| Medium | 557 (60.5) | 512 (60.6) | 45 (59.2) | 1.22 (0.54–2.32) | 0.536 |
| Low | 170 (18.4) | 152 (17.8) | 18 (23.7) | 1.67 (0.79–3.52) | 0.177 |
| **Clinical variables** | | | | | |
| **Gestational age (in weeks)** | | | | | |
| ≥ 37 | 832 (91.6) | 762 (91.5) | 70 (93.3) | 1.00 | |
| < 37 | 76 (8.4) | 71 (8.5) | 5 (6.7) | 0.77 (0.30–1.96) | 0.579 |
| **Birth weight (in grams)** | | | | | |
| 2.500–4.000 | 776 (87.5) | 712 (87.8) | 64 (86.5) | 1.00 | |
| < 2.500 | 78 (8.8) | 74 (9.1) | 4 (5.4) | 0.60 (0.21–1.70) | 0.337 |
| ≥ 4.000 | 31 (3.5) | 25 (3.1) | 6 (8.1) | 2.67 (1.06–6.75) | **0.038** |
| **Breast-feeding** | | | | | |
| Exclusive | 617 (67.0) | 569 (67.3) | 48 (63.2) | 1.00 | |
| Complemented | 220 (23.9) | 199 (23.6) | 21 (27.6) | 1.25 (0.73–2.14) | 0.414 |
| Formula milk | 84 (9.1) | 77 (9.1) | 7 (9.2) | 1.08 (0.47–2.47) | 0.859 |
| **Family history of diseases** | | | | | |
| Dyslipidemia | 148 (16.1) | 135 (15.6) | 13 (17.1) | 1.08 (0.58–2.03) | 0.797 |
| Obesity | 364 (39.5) | 311 (36.8) | 53 (69.7) | 3.96 (2.38–6.58) | **< 0.001** |
| Diabetes | 88 (9.6) | 73 (8.6) | 15 (19.7) | 2.60 (1.41–4.80) | **0.002** |

Legend: MW: minimum wage; CI: Confidence Interval

[a]p-value corresponding to the Pearson's chi-square test

**Table 2** Biochemical and genetics characteristics related to obesity in children and adolescents

| Characteristics | Total, *N* (%) | Non obese, *N* (%) | Obese, *N* (%) | Odds ratio, (CI 95%) | *p*-value [a] |
|---|---|---|---|---|---|
| **Biochemical variables** | | | | | |
| **Glycated hemoglobin** | | | | | |
| < 5.7 | 875 (95.5) | 807 (96.1) | 68 (89.5) | 1.00 | |
| ≥ 5.7 | 41 (4.5) | 33 (3.9) | 8 (10.5) | 2.87 (1.27–6.47) | **0.008** |
| **Total Cholesterol (mg/dL)** | | | | | |
| < 170 | 284 (31.0) | 261 (31.1) | 23 (30.7) | 1.00 | |
| ≥ 170 | 631 (69.0) | 579 (68.9) | 52 (69.3) | 0.77 (0.48–1.23) | 0.942 |
| **LDL-c (mg/dL)** | | | | | |
| < 110 | 553 (60.4) | 507 (60.4) | 46 (61.3) | 1.00 | |
| ≥ 110 | 362 (39.6) | 333 (39.6) | 29 (38.7) | 0.96 (0.59–1.55) | 0.868 |
| **HDL-c (mg/dL)** | | | | | |
| > 45 | 852 (93.1) | 787 (93.7) | 65 (86.7) | 1.00 | |
| ≤ 45 | 63 (6.9) | 53 (6.3) | 10 (13.3) | 2.28 (1.11–4.70) | **0.021** |
| **Triglycerides (mg/dL)[b]** | | | | | |
| Normal | 402 (43.9) | 383 (45.6) | 19 (25.3) | 1.00 | |
| Elevated | 513 (56.1) | 136 (16.0) | 56 (74.7) | 2.47 (1.44–4.22) | **0.001** |
| **Genetic variables** | | | | | |
| ***FTO* (rs1121980)** | | | | | |
| **Genotype** | | | | | |
| G/G | 289 (31.4) | 272 (32.2) | 17 (22.4) | 1.00 | |
| A/G | 483 (52.4) | 441 (52.2) | 42 (55.3) | 1.52 (0.85–2.79) | 0.154 |
| A/A | 149 (16.2) | 132 (15.6) | 17 (22.4) | 2.06 (1.01–4.20) | **0.040** |
| **Allele** | | | | | |
| G | 1061 (57.6) | 985 (58.3) | 76 (50.0) | 1.00 | |
| A | 781 (42.4) | 705 (41.7) | 76 (50.0) | 1.39 (1.00-1.94) | **0.047** |
| ***APOB* (rs693)** | | | | | |
| **Genotype** | | | | | |
| G/G | 411 (44.6) | 372 (44.0) | 39 (51.3) | 1.00 | |
| A/G | 403 (43.8) | 373 (44.1) | 30 (39.5) | 0.76 (0.46–1.26) | 0.296 |
| A/A | 107 (11.6) | 100 (11.8) | 7 (9.2) | 0.66 (1.26–1.48) | 0.339 |
| **Allele** | | | | | |
| G | 1225 (66.5) | 1117 (66.1) | 108 (71.1) | 1.00 | |
| A | 617 (33.5) | 573 (33.9) | 44 (28.9) | 0.79 (0.55–1.14) | 0.215 |
| ***NOS3* (rs1799983)** | | | | | |
| **Genotype** | | | | | |
| G/G | 528 (57.3) | 491 (58.1) | 37 (48.7) | 1.00 | |
| T/G | 342 (37.1) | 311 (36.8) | 31 (40.8) | 1.32 (0.80–2.17) | 0.270 |
| T/T | 51 (5.5) | 43 (5.1) | 8 (10.5) | 2.46 (1.08–5.63) | **0.027** |
| **Allele** | | | | | |
| G | 1398 (75.9) | 1293 (76.5) | 105 (69.1) | 1.00 | |
| T | 444 (24.1) | 397 (23.5) | 47 (30.9) | 1.45 (1.01–2.09) | **0.040** |
| **Genetic score[c]** | | | | | |
| 0 | 165 (17.9) | 157 (18.6) | 8 (10.5) | 1.00 | |
| 1 | 389 (42.2) | 358 (42.4) | 31 (40.8) | 1.70 (0.76–3.78) | 0.194 |
| 2 | 271 (29.4) | 250 (29.6) | 21 (27.6) | 1.65 (0.71–3.81) | 0.243 |
| 3 | 90 (9.8) | 76 (9.0) | 14 (18.4) | 3.61 (1.45–8.98) | **0.006** |
| 4 | 6 (0.7) | 4 (0.5) | 2 (2.6) | 9.81 (1.56–16.78) | **0.015** |

[a] p-value corresponding to the Pearson's chi-square test

[b]The cut-off point for triglycerides varies according to age, and is considered normal when the value is < 85 mg/dL for children (up to 9 years of age) and < 100 mg/dL for adolescents (between 10 and 17 years of age) (Brazilian Society of Cardiology, 2017). CI: Confidence Interval; HDL-c: High Density Lipoprotein Cholesterol;; LDL-c: Low Density Lipoprotein Cholesterol

[c]Risk allele score was created to evaluate the combined influence of polymorphisms in the *FTO* (rs1121980) and *NOS3* (rs1799983) genes on obesity. This score ranges from 0 (no risk alleles) to 4 (maximum risk alleles), based on the presence of protective or risk alleles. For *FTO*, allele G is protective and allele A is a risk allele. For *NOS3*, allele G is protective and allele T is a risk allele. Although the *APOB* (rs693) gene was analyzed, it was not included in the genetic risk score as it did not remain significant in the univariate analysis

**Table 3** Association between polymorphisms *FTO* and *NOS3* with obesity in childrens and adolescents

| SNPs | Genotype | Total, *N* (%) | Unadjusted OR (CI95%) | *p*-value[b] | Model adjusted 1[a] OR (CI95%) | *p*-value[b] | Model adjusted 2[a] OR (CI95%) | *p*-value[b] |
|---|---|---|---|---|---|---|---|---|
| ***FTO*(rs1121980)** | GG | 289 (31.4) | 1.00 | | 1.00 | | 1.00 | |
| | AG | 483 (52.4) | 1.52 (0.85–2.73) | 0.157 | 1.56 (0.87–2.81) | 0.149 | 1.53 (0.85–2.75) | 0.156 |
| | AA | 149 (16.2) | 2.06 (1.02–4.16) | **0.044** | 2.12 (1.05–4.32) | **0.041** | 2.07 (1.02–4.20) | **0.044** |
| ***NOS3*(rs1799983)** | GG | 528 (57.3) | 1.00 | | 1.00 | | 1.00 | |
| | TG | 342 (37.1) | 1.31 (0.80–2.16) | 0.287 | 1.29 (0.78–2.13) | 0.313 | 1.34 (0.81–2.22) | 0.252 |
| | TT | 51 (5.6) | 2.51 (1.10–5.73) | **0.029** | 2.61 (1.13–5.98) | **0.024** | 2.49 (1.08–5.73) | **0.032** |
| **Risk allele score** | (0–4) | 1.33 (1.27–1.39)* | 1.45 (1.13–1.89) | **0.004** | 1.46 (1.13–1.88) | **0.004** | 1.46 (1.13–1.89) | **0.004** |

Legend: OR: Odds ratio; CI: Confidence interval

[a]The directed acyclic graph (DAG) was used to support the theoretical model for the adjusted analysis between *FTO* and *NOS3* polymorphism genotypes (exposure) and obesity (outcome). Analysis adjusted for the following minimum and sufficient variables, according to two possible suggested models. Model 1: adjusted for skin color. Model 2: adjusted for age, birth weight, breastfeeding, family income, glycemic and lipid profile

Collinearity among variables in the adjusted model 2 evaluated by variance inflance factor (VIF) with the maximum remaining VIF = 1.2179

[b]p-value corresponding to the Wald test

*Data are presented as a mean and 95% confidence interval

Risk allele score was created to evaluate the combined influence of polymorphisms in the *FTO* (rs1121980) and *NOS3* (rs1799983) genes on obesity. This score ranges from 0 (no risk alleles) to 4 (maximum risk alleles), based on the presence of protective or risk alleles. For *FTO*, allele G is protective and allele A is a risk allele. For *NOS3*, allele G is protective and allele T is a risk allele. Although the *APOB* (rs693) gene was analyzed, it was not included in the genetic risk score as it did not remain significant in the univariate analysis

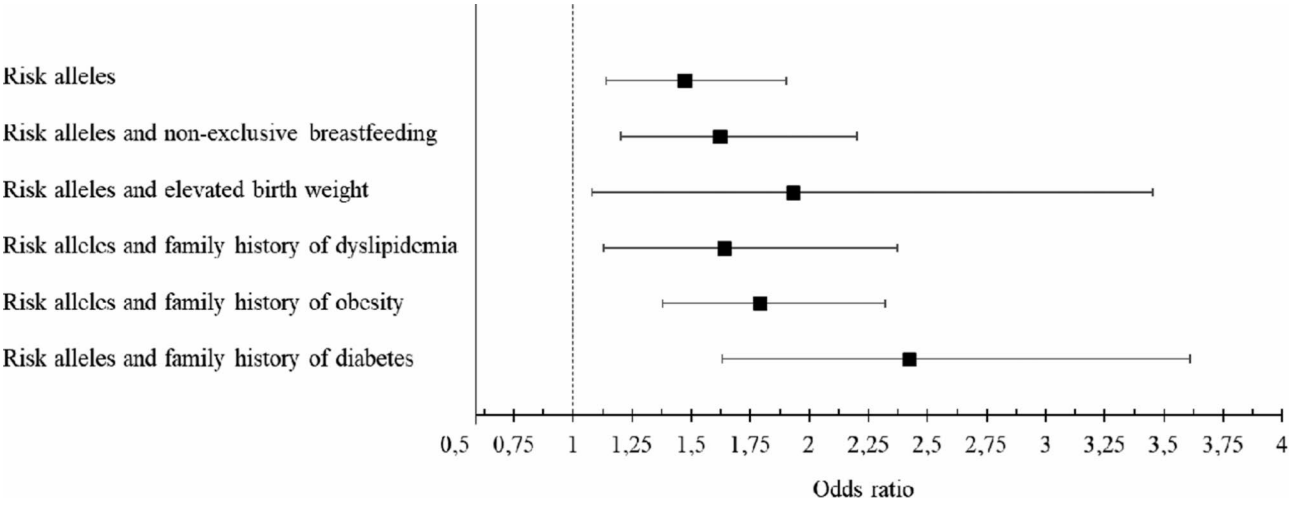


**Fig. 2** Modification of obesity risk based on the interaction between risk alleles and clinical factors.

respectively) remained significant, with both showing a more than a twofold increased likelihood of being obese.

In addition, the distribution of the genetic risk score showed that 17.9% of students had zero risk alleles, 42.2% had one risk allele, 29.4% had two risk alleles, 9.8% had three risk alleles, and only 0.7% carried four risk alleles (Table 2 ). When considering the sum of risk alleles from both SNPs as a continuous variable, for both models used, each additional risk allele increased the odds of the youth being obese by 1.4 times (Table 3 ). Moreover, individuals carrying 3 or 4 risk alleles had a markedly higher obesity prevalence compared to those with fewer risk alleles, reinforcing a potential cumulative genetic effect (Table 2 ).

The stratified analysis by age revealed that the associations between SNPs and obesity were stronger among adolescents (10–17 years) than among children (6–9 years). In the younger age group, the reduced sample size resulted in wider confidence intervals, indicating lower estimate precision. Despite these differences, the overall association patterns remained consistent in both groups. Full results of the stratified analyses are provided in the Supplementary Material (Table S2 and S3).

Figure 2 illustrates the modification of obesity risk on the basis of the interaction between risk alleles and clinical factors. Each additional risk allele significantly increased the likelihood of obesity in the presence of specific clinical conditions. Specifically, for children and

adolescents who were not exclusively breastfed, the addition of one risk allele increased the likelihood of obesity by 62% [OR = 1.62 (CI: 1.20–2.20)]. For those with a high birth weight, each additional risk allele increased the likelihood of obesity by 93% [OR = 1.93 (CI: 1.08–3.45)]. Similarly, each additional risk allele was associated with a 64% increase in the likelihood of obesity in individuals with a family history of obesity [OR = 1.64 (CI: 1.13–2.37)], a 79% increase in those with a family history of DM2 [OR = 1.79 (CI: 1.38–2.32)], and a 142% increase in those with a family history of dyslipidemia [OR = 2.42 (CI: 1.63–3.61)].

## Discussion

The present study was one of the first to evaluate the combination of SNPs in the *FTO* and *NOS3* genes in association with obesity in children and adolescents from Ouro Preto, Brazil. Data from the last few decades indicate that pediatric obesity is associated with numerous complications and comorbidities, and as its prevalence has increased, diseases traditionally seen in adulthood have become more common early in life [36–38]. In Brazil, obesity has also significantly increased among children and adolescents [39]. On the basis of a previous study conducted 15 years ago within the same target population [24], this trend among youth has persisted, increasing from 6.7 to 8.5%.

In Brazil, studies involving children and adolescents that have investigated the SNPs rs1121980 and rs1799983 and their associations with obesity are scarce. The present study revealed that for each additional risk allele of these SNPs, the chance of obesity increased by 1.46 times ($p = 0.004$). The frequencies of the risk allele A at rs1121980 and the risk allele T at rs1799983 were 42.4% and 24.1%, respectively. Considering this frequency within the group of obese children and adolescents, allele A was significantly different from that in the eutrophic group (50.0% versus 41.7%, $p = 0.047$). Similarly, the allele T also exhibited a significant difference between the obese and eutrophic groups, with frequencies of 30.9% and 23.5%, respectively ($p = 0.040$). These findings align with those of several studies in the literature [2, 5, 6, 12].

The presence of the A allele of the SNP rs1121980 may affect the transcriptional activity of the *FTO* gene, resulting in increased BMI, WC, and extreme obesity [2, 5, 6]. Its presence was associated with Grade III obesity in adults (BMI > 40) [2], and with higher BMI (increase of 0.31 units per allele; $p < 0.001$) and larger WC (increase of 0.77 cm per allele; $p < 0.001$) [6]. Therefore, the data from the present study reinforce these findings.

Studies indicate that decreased bioavailability of nitric oxide is also involved in the pathophysiology of metabolic diseases, such as obesity and DM2 [40, 41]. Therefore, the contribution of *NOS3* gene SNPs, particularly the T allele at rs1799983, to susceptibility to these conditions has been investigated. Consistent with our data, Podolski et al. (2007) reported that among African and Euro-American youth, carriers of the T allele of the same SNP presented increased BMI and WC [12]. Another study reported that, in addition to the T allele (OR = 1.72; $P = 0.001$), the TT genotype in North Africans was associated with a nearly threefold increased risk of developing obesity (OR = 2.93; $P = 0.03$) [40]. These findings reinforce the suggestion that this SNP may influence genetic susceptibility to obesity. What remains to be investigated is whether this relationship is plausible in children and adolescents, as few studies, particularly in Brazil, have evaluated this SNP and obesity in young populations.

Our study focused on clinical factors that may also interact with the aforementioned SNPs in the development of obesity. Regarding neonatal history, those born with excess weight (≥ 4,000 g) were found to have 2.67 (CI 1.06–6.75, $p = 0.038$) times higher odds of obesity compared to those born with an appropriate weight (between 2,500 and 4,000 g), which aligns with several other studies [22, 42–44]. In interaction with the risk alleles assessed in this study, the odds of obesity were 1.93 times greater for individuals with this profile.

Zhu et al. (2023) reported that for children over 8 years of age, inadequate free play time was a key factor in childhood obesity, whereas for those under 8 years of age, birth weight and parental BMI were significant contributors [44]. The current study also revealed that students with a family history of obesity were more likely to be obese [OR 3.96, $p < 0.0001$] than were students without a family history of obesity. Additionally, other studies have reported this relationship [45–47], with a significantly greater risk if both parents are obese [OR 6.83, $p < 0.05$] than when only one parent is obese [OR 2.49, $p < 0.05$] [47]. Furthermore, if the parents had severe obesity (BMI ≥ 35), the risk increased to 22.34 ($p < 0.01$), regardless of age, sex, socioeconomic status, and ethnicity [45]. Few studies have focused on rs1121980 and a family history of obesity. In Brazil, Todendi et al. (2020) reported that parental weight also contributed to obesity and additionally interacted with the *FTO* gene, which supports our findings [48].

The relationships between a family history of diabetes and dyslipidemia and obesity development are complex and involve various factors. In addition to genetics, families with these conditions tend to have poorer eating behaviors, creating an obesogenic environment that promotes overweight and obesity in children [49].

Finally, we observed that children and adolescents carrying the risk alleles of both SNPs who were not exclusively breastfed had 1.62 times greater odds of obesity ($p = 0.002$). The protective effect of breastfeeding is attributed to slower growth rates than those of formula

feeding [50, 51], with significant differences in bioactive nutrient composition influencing endocrine modulation [52]. Further studies are recommended to explore this relationship.

This study highlights the importance of assessing genetic predisposition to obesity in a representative sample of children and adolescents. This is one of the first studies to find a significant association between childhood obesity and the SNPs rs1121980 and rs1799983 in the Brazilian population, both individually and together. Given the scarcity of genetic studies in pediatric populations, these findings may enhance the understanding of pathophysiological mechanisms and aid in the development of new prognostic and therapeutic tools for obesity.

While our findings offer valuable insights, there are limitations to the study. The cross-sectional design prevents causal inferences between exposure factors and obesity. Additionally, we did not assess key factors such as physical activity levels, dietary patterns, or pubertal stage. It is also important to point out that the blood collection without fasting may have influenced the high triglyceride levels, as it was not possible to obtain information about the previous meal, so it was not possible to assess the fat load ingested. Although stratification by age provided useful insights into potential differences between children and adolescents, it reduced the sample size in each group, leading to lower statistical power and wider confidence intervals, particularly in the 6–9-year-old group. Finally, the potential impact of the pandemic on BMI and biochemical parameters should be considered, although it remains unclear whether these effects are temporary or permanent.

In conclusion, our results suggest that rs1121980 and rs1799983 may influence obesity development in Brazilian children and adolescents in urban settings. Given the heterogeneity of the literature and the complex interplay between genes and the environment, we recommend further studies to explore the physiological effects of allelic variations in the *FTO* and *NOS3* genes, particularly rs1121980 and rs1799983, in relation to environmental factors.

#### Abbreviations

| | |
|---|---|
| SNPs | Single nucleotide polymorphisms |
| FTO | Alpha-ketoglutarate-dependent dioxygenase |
| G | Guanine |
| A | Adenine |
| BMI | Body mass index |
| WC | Waist circumference |
| NOS3 | Endothelial nitric oxide |
| DM2 | Diabetes mellitus type 2 |
| TC | Total cholesterol |
| TG | Triglyceride |
| LDL | Low-density lipoprotein |
| HbA1c | Glycated hemoglobin |
| HDL | High-density lipoprotein |
| DAG | Directed acyclic graph |
| APOB | Apolipoprotein B |
| HWE | Hardy–Weinberg equilibrium |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-025-05570-3.

Supplementary Material 1

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#### Author contributions

T.S.V. and G.L.L.M.C. conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.A.P.B. carried out the biochemical and molecular analyses, drafted the initial manuscript and critically reviewed the manuscript for important intellectual content.L.A.A.M.J. carried out the statistical analyses, drafted the figures and tables and critically reviewed the manuscript for important intellectual content.L.G.L., W.W.O., C.B.C., A.C.M.C., G.T.A., D.F.A.R., I.M.C.R., A.V.C.R., J.P.M.M. field data collection and manuscript review for important intellectual content.All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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#### Data availability

The datasets generated and/or analyzed as part of the current study are not publicly available due to confidentiality agreements with subjects. However, they can be made available solely for the purpose of review and not for the purpose of publication from the corresponding author upon reasonable request.

## Declarations

#### Ethics approval and consent to participate

This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Federal University of Ouro Preto (CAAE 28680020.0.0000.5150).

#### Consent for publication

Informed consent was obtained from all individual participants included in the study, and written informed consent was obtained from the parents.

#### Competing interests

The authors declare no competing interests.

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## Publisher's note