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The polymorphism rs2293855 MTMR9 gene and genetic score are associated with higher atherogenic risk in Brazilian schoolchildren

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41 *ABSTRACT*

42

43 *Purpose:* Evaluate the associations of *FTO* (rs9939609), *MC4R* (rs17782313), *MTMR9* (rs2293855), and
44 the genetic score with atherogenic risk in Brazilian children.

45 *Methods:* This is a cross-sectional study conducted with 544 children aged 4 to 9 years old. We obtained
46 sociodemographic and lifestyle data by questionnaires, and biological sample (DNA) through oral *swab*.
47 The single nucleotide polymorphisms (SNP) *FTO* (rs9939609), *MC4R* (rs17782313), and *MTMR9*
48 (rs2293855) were identified by the system taqman SNP genotyping and evaluated the obesity-related
49 genetic risk score. Blood samples were collected for the lipid profile analysis (serum total cholesterol, HDL-
50 c, LDL-c, triglycerides). The atherogenic indexes (Castelli I and II indexes, atherogenic coefficient - AC,
51 lipoprotein combination index - LCI and plasma atherogenic index - AIP) were calculated. We compared
52 the distributions of outcomes (lipid profile and atherogenic indexes) by genotype categories using
53 multivariable linear regression.

54 *Results:* Children with AG/AA genotypes in the polymorphism *MTMR9* (rs2293855), had lower HDL-c
55 level and higher TC/HDL-c, LDL-c/HDL-c ratios, and AC. Those with one or more polymorphisms (*FTO*
56 rs9939609, *MC4R* rs17782313, and *MTMR9* rs2293855) in the genetic risk score had lower HDL-c and
57 higher TC/HDL-c, AC, LCI, and AIP.

58 *Conclusion:* The risk allele (AG/AA) of the *MTMR9* (rs2293855) and the higher obesity-related genetic
59 risk score were positively associated with higher atherogenic risk in Brazilian children.

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61 *Key-words:* children, single nucleotide polymorphism, dyslipidemia, cardiovascular disease.

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81 INTRODUCTION

82 Cardiovascular diseases (CVD) are responsible annually for the death of 17.9 million people in
83 the world [1], and the dyslipidemia is an important risk factor for its occurrence [2], that is characterized
84 by the increase in the concentration of lipids or lipoproteins in the blood. The dyslipidemia can be
85 influenced by environmental and genetic factors [3], being the latter responsible for about 40% of this
86 occurrence [4].

87 The association of increased levels of total cholesterol (TC), low-density lipoprotein cholesterol
88 (LDL-c), triglycerides (TG), and low values of high-density lipoprotein cholesterol (HDL-c) are associated
89 with the risk for CVD in children [5,6,7]. However, it is necessary to evaluate atherogenic indexes, such as
90 the LDL/HDL and TC/HDL ratios, atherogenic plasma index (AIP), atherogenic coefficient (AC) and the
91 lipoprotein combined index (LCI), once that they have a greater predictive capacity for cardiovascular risk
92 than isolated traditional markers [8,9].

93 The knowledge of the gene-disease relationship is of great importance, once it allows early action
94 in the prevention and treatment of pathologies, and also, because we can present different responses when
95 the same intervention occurs [10]. In adults, the relationship between the genes *Fat Mass and Obesity*
96 *Associated (FTO)* rs9939609, *Myotubularin-related protein 9 (MTMR9)* rs2293855 [11] and *Melanocortin-*
97 *4 receptor (MC4R)* rs2229616 [12] and dyslipidemia is already presented. Others studies with adults
98 identified that carriers of the *FTO* rs9939609 and *MC4R* rs12970134 A-allele showed changes in the lipid
99 profile, with a decrease of HDL-c, and increase of TG, TC and AIP [13,14]. In children, no relationship
100 was found between *FTO* rs993960 and the alterations in the lipid profile [15]. We did not find studies
101 evaluating the association of polymorphisms in the *MC4R* and *MTMR9* genes with dyslipidemia in
102 childhood.

103 Since childhood dyslipidemia is associated with cardiovascular diseases in adulthood, and early
104 diagnosis and preventive actions can reduce heart diseases' mortality in adults [16], we highlight the
105 importance to study of the gene-dyslipidemia relationship in young ages. Considering the absence of
106 studies, mainly in relation to the *MC4R* and *MTMR9* genes, that evaluated the occurrence of genetic
107 polymorphisms related with lipid markers and atherogenic indexes in childhood [11,17], the objective of
108 this study was to evaluate the association of the *FTO* (rs9939609), *MC4R* (rs17782313), *MTMR9*
109 (rs2293855) polymorphisms and the obesity-related genetic risk score with atherogenic risk in Brazilian
110 children. We hypothesize is that these alleles are associated with higher atherogenic risk.

111

112 MATERIALS AND METHODS

113 *Participants and study design*

114 This is a cross-sectional study conducted with 544 children aged from 4 to 9 years old, in the city
115 of Viçosa, Minas Gerais, Brazil, as part of the Schoolchildren Health Assessment Survey (PASE, *in*
116 *portuguese*) and the Lactation Support Program (PROLAC, *in portuguese*) accomplished in 2015 and
117 2016.

118 The sample size calculation and the sampling process of PASE [18,19] and PROLAC [20] have
119 been described previously. Figure 1 presents the sampling process of the children participating in this study.

120 All the children were reevaluated in 2018, with measurements of weight and height, body mass index
 121 calculation, and the genetic material collected through an oral swab.
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123
 124 Figure 1. Sampling process flowchart of the study.
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126 The sample size power of the study was calculated by adopting the means and standard deviations
 127 of the TC in the group without the risk allele of the *MTMR9* rs2293855 (GG/AG) (155.7 ± 26.2) and with
 128 the risk allele (AA) (158.2 ± 33.3) by the OpenEpi *software*. The statistical significance level was 5%, and
 129 the sample size had power $\geq 80\%$. The calculation of the sample size was performed for all polymorphisms
 130 and the *MTMR9* rs2293855 was the one with the lowest statistical power.

131 This study was developed according to the guidelines established in the Declaration of Helsinki
 132 and approved by the Human Research Ethics Committee of the Federal University of Viçosa (UFV, in
 133 Portuguese) (protocols numbers 663.171/2014, 892476/2014 and 2.832.590/2018). All parents and children
 134 were informed about the study's purpose and all guardians of the children signed the Informed Consent
 135 Form.

136 137 Laboratory Methods

138 All evaluations were done at the Laboratory of Clinical Analysis in UFV. Blood samples were
 139 collected intravenously after 12 hours of fasting.

140 The lipid profile (TG, TC, LDL-c, and HDL-c) was quantified by automatic equipment
 141 (BioSystems 200 Mindray® model, Nanchan, China) according to the recommendations of the kit
 142 manufacturer (Bioclin®, Belo Horizonte, MG, Brazil). Children with at least one of the following altered
 143 lipids were classified with dyslipidemia [21]: TC ≥ 170 mg/dL, HDL-c < 45 mg/dL, LDL-c ≥ 110 mg/dL,
 144 TG ≥ 75 mg/dL.

145 Moreover, we calculated atherogenic indexes such as the Castelli risk index I (TC/HDL-c), Castelli
 146 index risk II (LDL-c/HDL-c), atherogenic index of plasma (AIP), atherogenic coefficient (AC), and

lipoprotein combine index (LCI). The non-HDL-c was estimated by the equation: (TC - HDL-c). AIP was defined as the base 10 logarithms of the TG/HDL-c ratio. AC and LCI were defined as non-HDL-c/HDL-c and (TC x TG x LDL)/HDL-c ratios, respectively [22-24].

150

151 *Single Nucleotide Polymorphisms Genotyping*

152 In the collection of buccal cells was performed with circular movements, 30 times on each side on
153 the inner face of both cheeks with a sterilized cytological brush. The brushes were placed in absolute ethanol
154 and storage on 4° C refrigerator until the moment of DNA extraction. Genomic DNA was extracted from
155 buccal epithelial cells following a modified protocol of Miller, Dykes, Polesky (1988) [25] and were kept
156 at -20 degrees. The concentration and purity of the DNA was determined by spectrophotometry using
157 NanoDrop 2000c. DNA concentration was evaluated at 260 nm and the ratio of readings at 260 nm and 280
158 nm was used to estimate the DNA purity.

159 The chosen SNPs were genotyped using the real-time PCR TaqMan assay utilizing two
160 differentially fluorescently labeled probes that permitted the detection of both alleles in a single reaction
161 (Applied Biosystems INC, Foster City, CA, USA) [26]. The PCR primers and Taqman probes had been
162 previously developed at Applied Biosystems for each SNP studied. Assays were performed on an ABI 7900
163 HT Fast PCR Real Time System (Applied Biosystems, Foster City, CA), and the genotype assignments
164 were conducted using the TaqMan™ Genotyper Software, in a 384-well format and using manufacturer's
165 instructions. The commercial classifications of the TaqMan™ SNP Genotyping methodology test used by
166 ABI specific for the study of the *FTO* site is C__30090620_10, locus *MC4R* is C_32667060_10 and locus
167 *MTMR9* is C__16185295_10. The Taq-Man™ SNP *Genotyping methodology* was based on using a pre-
168 designed assay and validated by the company *Applied Biosystems* (ABI).

169 The obesity-related genetic risk score was calculated using the genotype information according to
170 the allelic composition of the three SNPs studied [27]. The genotypes were coded zero, 1, 2 and 3, creating
171 a score, adding the numbers of genotypes of each study participant. The children received a
172 score "zero" when presented no risk allele (TT, TT, GG), "1" when presented one risk allele in the *FTO*
173 rs9939609, *MC4R* rs17782313 or *MTMR9* rs2293855 gene, "2" when presented the polymorphism of two
174 genes, and "3" when presented all polymorphisms. After, the children were divided into two groups, those
175 that did not have any polymorphism and those that had one or more genetic polymorphisms.

176

177 *Sociodemographic, economic, and lifestyle characteristics*

178 A semi-structured questionnaire was applied, pre-coded, and pre-tested in a pilot study by the
179 researchers, containing variables such as sex and age of the children, maternal education, and household's
180 per capita income.

181 Sedentary behavior was assessed using screen time, including television, video game, computer,
182 cell phone, and tablets. According to the American Academy of Pediatrics (2013) [28], the child who had
183 a screen time higher than 2 hours per day was classified as sedentary.

184

185 *Anthropometry and body composition*

Weight and height measurements were performed in all children. Weight was measured using an electronic digital scale (Tanita®, model BC 553, Arlington Heights, IL, USA), with a capacity of 150kg and a sensitivity of 50g. Children were weighed without shoes and wearing light clothes. Height was measured using a vertical anthropometer (Altuxata®, Belo Horizonte, MG, Brazil), divided into centimeters and subdivided into millimeters, according to the standards recommended by Jelliffe (1968) [29].

Body composition was evaluated by Dual Energy X-ray Absorptiometry (DXA). The examination was carried out at the Health Division of the Federal University of Viçosa. This equipment is considered a reference method for assessing body composition and, due to the low radiation level, it does not pose any health risk [30]. During the scanning, the child remained in a supine position, and the protocol was established for the child to be in light clothing, without earrings, bracelets, or any metal adornment.

Data analyses

Exposures. Genotypes polymorphisms (*FTO* rs9939609, *MC4R* rs17782313, *MTMR9* rs2293855, and genetic risk score) as categorical variables.

Outcomes. Lipid markers (TC, LDL-c, HDL-c, TG, and non-HDL-c) and atherogenic indexes (Castelli risk indexes I and II, AC, LCI, and AIP) as continuous variables.

Covariates. We considered as covariates the child's sex, height- and BMI-for age Z-score, body fat, screen time, the household's per capita income, maternal education, and total energy intake. Height- and BMI-for-age Z-score were calculated according to the sex- and age-specific reference for children and adolescents [31].

Statistical analyses. The normality of the variables was assessed by the Kolmogorov-Smirnov test. We evaluated the distributions of outcomes (lipid profile and atherogenic indexes) using mean $\pm$ standard deviation (SD) according to sociodemographic, anthropometric, body composition, and lifestyle characteristics. Then, we compared the distributions of outcomes (lipid profile and atherogenic indexes) by genotype categories (exposure), using mean differences and 95% confidence intervals (CI) from unadjusted and multivariable linear regression models adjusted by sex, age, screen time, per capita income, maternal education, body fat, and total energy intake. The adjustment variables were selected based on the literature review, and robust estimates of the variance were specified in all models, which are consistent to heteroscedasticity and normality [32]. Analyses were carried out in the Stata® version 14 (StataCorp LP, College Station, TX, USA). The significance level was 5% for all hypothesis tests.

The Hardy-Weinberg balance was assessed by the chi-square test, checking the balance in the genotypic frequencies of the *FTO* (rs9939609), *MC4R* (rs17782313), *MTMR9* (rs2293855) on the studied populations.

RESULTS

A total of 544 children were evaluated in this study, the mean $\pm$ SD age of participants was 7.2 $\pm$ 1.6 years old; 51.3% were girls, and 55.5% presented dyslipidemia. We observed that 32.4% did not show the risk allele (TT), 49.6% had a risk allele (AT) and 18% presented two risk alleles (AA) of the *FTO* polymorphism. In relation to the *MC4R* 61.5% did not show the risk allele (TT), 33.8% had a risk allele

(TC) and 4.7% presented two risk alleles (CC) of the *MC4R* polymorphism. 45% of children did not show the risk allele (GG), 45.1% had a risk allele (AG) and 9.9% presented two risk alleles (AA) of the *MTMR9* polymorphism. Children with over one or two polymorphisms in the obesity-related genetic risk score were 28.5% and 2.2%, respectively. Genotypic proportions were in Hardy-Weinberg equilibrium.

Height-for-age Z-score was negatively associated with LDL-c and LDL-c/HDL-c. BMI-for-age Z-score and body fat were negatively associated with HDL-c, and positively associated with TG, LCI, and AIP. In addition, BMI-for-age was positively associated with TC/HDL-c and AC. Per capita income and maternal education were positively associated with LDL-c, TG, LCI, and AIP. Moreover, maternal education was positively associated with TC, non-HDL-c, TC/HDL-c, LDL-c/HDL-c, and AC, (Table 1).

We did not find associations of the risk alleles *FTO* rs9939609 and *MC4R* rs17782313 with the atherogenic risk (Tables 2 and 3).

After the adjustment for potential confounders, the risk alleles of the *MTMR9* rs2293855 was positively associated with lipid markers and atherogenic risk. Individuals with AG/AA genotypes in the *MTMR9* rs2293855 were related to -1.75 (95% CI: -3.12, -0.38) units lower of HDL-c; and to 0.15 (95% CI: 0.01, 0.29), 0.13 (95% CI: 0.01, 0.25), and 0.15 (95% CI: 0.01, 0.29) unit higher of TC/HDL-c, LDL-c/HDL-c and AC, respectively, in comparison with children without polymorphism (Table 4).

In the same way, after adjustment, the genetic risk score was negatively associated with HDL-c and positively associated with the TC/HDL-c, AC, LCI and AIP. Children who had one or more polymorphisms were related to -2.11 (95% CI: -4.08, -0.14) units lower of HDL-c; and to 0.16 (95% CI: 0.02, 0.31), 0.16 (95% CI: 0.02, 0.31), 4.36 (95% CI: 0.30, 8.36), 0.04 (95% CI: 0.004, 0.08) units higher of TC/HDL-c, AC, LCI, and AIP, respectively, in comparison with children that presented null obesity-related genetic risk score (Table 5).

DISCUSSION

In this cross-sectional study with Brazilian children, we confirmed the hypothesis that the risk allele of the *MTMR9* rs2293855 polymorphism and the obesity-related genetic risk score were positively associated with higher atherogenic risk. Despite the prevalence of the *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms in this study being similar to other studies [33-36], we did not find associations of these risk alleles with the atherogenic risk in children. It is also noteworthy the high prevalence of dyslipidemia among children in this study.

To our knowledge this is first study identify a direct relationship between the *MTMR9* rs2293855 polymorphism with the lipid markers and atherogenic indexes (HDL-c, TC/HDL-c, LDL-c/HDL-c, and AC) in schoolchildren. This association has been demonstrated previously, but in a study with Japanese adults, that found positive association between *MTMR9* rs2293855 polymorphism and metabolic syndrome, including alterations in markers of dyslipidemia, hypertension and fasting glucose [11].

Although the mechanisms of action of the *MTMR9* gene on lipid profile markers are not clear, it is believed that this association is complex and mediated by changes in adiposity and blood glucose. The *MTMR9* gene affects glucose transport through the dephosphorylation of phosphatidylinositol 3-phosphate (PtdIns3P) [17,37]. Individuals with *MTMR9* rs2293855 polymorphism presented higher fasting glucose, glucose intolerance, reduced insulin sensitivity and increased risk of prediabetes [37]. Still, Yanagiya et al.

(2007) [17] when evaluating an association of the *MTMR9* rs2293855 polymorphism with obesity, identified an association between genetic polymorphism and higher BMI values. It is known that excess adiposity, especially in the visceral region, is associated with insulin resistance and this hormone has an antilipolytic effect. Therefore, changes in insulin performance can increase triglyceride hydrolysis, with consequent excessive release of free fatty acids into the circulation and change in lipid profile markers [38,39]. It is also noteworthy that some key enzymes involved in HDL metabolism, such as cholesterol ester transfer protein (CETP), lecithin/cholesterol acyltransferase (LCAT), liver lipase (HL) and protein phospholipid transfer protein (PLTP) are altered in people with obesity that has insulin resistance [40].

It is noteworthy that, like any gene expressed in the hypothalamus, *MTMR9* plays a role in the regulation of hypothalamic peptides, regulating appetite and influencing food preferences [17]. It is speculated that individuals with the polymorphism in the *MTMR9* gene would have a greater preference for fatty foods, thus contributing to the occurrence of dyslipidemia.

We did not find associations of *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms with dyslipidemia, which corroborates with other studies [41-43]. It is known that the *FTO* and *MC4R* gene are expressed in different ways in the individual, depending on their age. The same variant associated with obesity and insulin resistance in adults did not show significant differences among children [44-46], that is in accordance with our sample.

Our findings showed that the obesity-related genetic risk score was positively associated with the lipid markers and atherogenic indexes (HDL-c, TC/HDL-c, AC, LCI, AIP). A review study identified that genetic risk scores have been a way of aggregating multiple sets of results into a single genetic predictor for cardiovascular diseases [47]. It is important to consider the interaction of genes in our body [48], and studies have identified a higher predictive value of the genetic risk score for cardiovascular risk when compared to a single genetic variant worked in isolation [49,50].

Several studies have shown the relationship between the *FTO* rs9939609 [51,52] and *MTMR9* rs2293855 [17] polymorphisms with weight excess in adults, and between *MC4R* rs17782313 polymorphisms with obesity in children [53]. Despite knowing that adipose tissue plays a central role in maintaining lipid homeostasis [54], we could evidence the relationship of *MTMR9* rs2293855 polymorphism and the genetic risk score with higher atherogenic risk in schoolchildren, even after adjusting for body fat.

Although the power of this study is higher than 80%, it presents a limitation that our data was not representative of the city of Viçosa, nor of Brazilian population, so the results generalization should be avoided. However, several strengths need to be highlighted. To our knowledge, this is the first study to evaluate the association of the *MTMR9* rs2293855 polymorphism and genetic risk score with the lipid markers and non-traditional atherogenic indexes which, these last, according to the literature, have a higher predictive value for cardiovascular risk [8].

We conclude that the risk allele of the *MTMR9* rs2293855 and the obesity-related genetic risk score were positively associated with higher atherogenic risk in Brazilian children. More attention should be given to evaluating risk allele to better understand the relation between genotype and alterations of the lipid profile even under the age of 10 years old.

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516 The authors declare no conflicts of interest.

517

518 *Authorship*

519 M.A.S assisted the conception and design of this work, analysis and interpretation of the data,
520 conducted the literature search, as well as wrote the manuscript. C.M.M.R. assisted the conception and design
521 of this work, revised and approved the final version to be published. M.B.L. assisted data collection, revised
522 and approved the final version to be published. S.A.V.R. revised and approved the final version to be published.
523 S.C.C.F. assisted data collection, revised and approved the final version to be published. S.C.C.F. revised and
524 approved the final version to be published. J.G.V.N designed the study including the data collection,
525 coordinated, supervised and approved the final version to be published. J.I.A.L designed the study including
526 the data collection, coordinated, supervised and approved the final version to be published. D.F.D designed the
527 study including the data collection, coordinated, supervised and approved the final version to be published.
528 J.F.N designed the study including the data collection, coordinated, supervised and approved the final version
529 to be published.

530

531 *Ethics approval*

532 This study was developed according to the guidelines established in the Declaration of Helsinki
533 and approved by the Human Research Ethics Committee of the Federal University of Viçosa (UFV, in
534 Portuguese) (protocols numbers 663.171/2014, 892476/2014 and 2.832.590/2018).). All parents and

535 children were informed about the study's purpose and all guardians of the children signed the Informed
536 Consent Form.

Table 1. Distribution of lipid markers and, atherogenic indexes according to sociodemographic, anthropometric, body composition and lifestyle characteristics in children from Viçosa, Minas Gerais, Brazil, 2015.

Characteristics	N	Total cholesterol (mg/dL)		HDL-c (mg/dL)		LDL-c (mg/dL)		Triglyceride (mg/dL)		Non-HDL-c (mg/dL)	
		Mean $\pm$ SD	<i>P</i> value	Mean $\pm$ SD	<i>P</i> value	Mean $\pm$ SD	<i>P</i> value	Mean $\pm$ SD	<i>P</i> value	Mean $\pm$ SD	<i>P</i> value
Sex											
Female	265	157.82 $\pm$ 27.08	0.37	49.74 $\pm$ 10.26	0.55	91.11 $\pm$ 24.06	0.23	70.56 $\pm$ 28.94	0.09	107.69 $\pm$ 25.23	0.22
Male	279	155.73 $\pm$ 27.30		50.28 $\pm$ 10.94		93.59 $\pm$ 23.66		75.21 $\pm$ 34.18		105.45 $\pm$ 25.00	
Height-for-age (Z-score)											
-1 to < 0	186	157.80 $\pm$ 26.28	0.28	49.47 $\pm$ 10.48	0.63	94.13 $\pm$ 22.51	0.02*	70.05 $\pm$ 27.83	0.08	107.75 $\pm$ 24.69	0.05
0 to 1	206	157.50 $\pm$ 29.12		50.34 $\pm$ 10.48		93.09 $\pm$ 25.77		71.48 $\pm$ 32.17		107.16 $\pm$ 26.97	
> 1	152	154.65 $\pm$ 25.55		50.20 $\pm$ 10.92		89.29 $\pm$ 22.55		78.44 $\pm$ 35.14		104.44 $\pm$ 23.00	
BMI-for-age (Z-score)											
< -2	81	156.34 $\pm$ 32.10	0.38	48.34 $\pm$ 9.75	0.01*	95.97 $\pm$ 29.27	0.74	69.60 $\pm$ 27.94	<0.0001*	108.00 $\pm$ 30.11	0.63
-2 to 1	306	157.72 $\pm$ 26.30		51.30 $\pm$ 10.96		91.87 $\pm$ 22.66		67.87 $\pm$ 25.73		106.07 $\pm$ 23.78	
1 to 2	97	157.31 $\pm$ 25.32		49.78 $\pm$ 10.57		92.90 $\pm$ 23.16		79.37 $\pm$ 37.96		107.53 $\pm$ 24.53	
> 2	60	151.93 $\pm$ 27.46		46.00 $\pm$ 8.47		89.28 $\pm$ 22.71		92.85 $\pm$ 43.00		105.93 $\pm$ 25.91	
% Body fat (terciles)											
$\leq$ 15.7	178	153.65 $\pm$ 27.93	0.89	49.48 $\pm$ 10.35	0.04*	90.86 $\pm$ 25.72	0.81	67.28 $\pm$ 26.84	<0.0001*	104.17 $\pm$ 25.86	0.52
15.7 to 24.4	175	158.95 $\pm$ 27.24		51.78 $\pm$ 10.81		93.19 $\pm$ 23.76		67.62 $\pm$ 25.30		107.16 $\pm$ 24.31	
> 24.4	191	157.78 $\pm$ 26.28		48.85 $\pm$ 10.44		93.05 $\pm$ 22.11		83.14 $\pm$ 38.37		108.35 $\pm$ 25.12	
Screen time (hours/day) ¹											
$\leq$ 2	241	155.36 $\pm$ 25.38	0.07	49.37 $\pm$ 9.74	0.26	91.60 $\pm$ 23.12	0.12	72.55 $\pm$ 33.12	0.75	105.54 $\pm$ 24.27	0.14
> 2	303	157.95 $\pm$ 28.5		50.50 $\pm$ 11.21		93.00 $\pm$ 24.43		73.25 $\pm$ 30.74		107.44 $\pm$ 25.79	
Per capita income (US\$) [†]											
< 90.1 (1° tercile)	182	157.33 $\pm$ 25.19	0.09	49.33 $\pm$ 11.12	0.38	92.69 $\pm$ 21.94	0.02*	67.74 $\pm$ 30.60	0.004*	108.00 $\pm$ 23.13	0.11
90.1 to 157.8 (2° tercile)	181	154.88 $\pm$ 27.11		49.29 $\pm$ 9.93		90.57 $\pm$ 25.37		75.13 $\pm$ 33.24		105.59 $\pm$ 25.66	
> 157.8 (3° tercile)	181	158.29 $\pm$ 28.98		51.35 $\pm$ 10.67		93.94 $\pm$ 23.93		75.45 $\pm$ 30.92		106.36 $\pm$ 26.37	
Maternal education (years)											
$\leq$ 4	66	153.75 $\pm$ 24.36	0.01*	50.37 $\pm$ 9.60	0.96	86.20 $\pm$ 20.85	<0.0001*	69.83 $\pm$ 31.29	0.02*	103.37 $\pm$ 20.98	<0.0001*
5 to 10	168	153.10 $\pm$ 25.59		49.51 $\pm$ 10.10		88.81 $\pm$ 23.04		71.32 $\pm$ 34.88		103.58 $\pm$ 24.21	
11	210	157.89 $\pm$ 28.33		49.86 $\pm$ 11.63		94.66 $\pm$ 24.28		72.40 $\pm$ 28.65		108.02 $\pm$ 25.12	
$\geq$ 12	98	163.14 $\pm$ 28.07		50.78 $\pm$ 9.50		98.02 $\pm$ 24.77		79.67 $\pm$ 32.56		111.21 $\pm$ 28.51	

Continuation.

Characteristics	N	TC/HDL-c		LDL-c/HDL-c		AC		LCI		AIP	
		Mean $\pm$ SD	P value	Mean $\pm$ SD	P value	Mean $\pm$ SD	P value	Mean $\pm$ SD	P value	Mean $\pm$ SD	P value
Sex											
Female	265	3.5 $\pm$ 0.68	0.29	1.89 $\pm$ 0.62	0.27	2.25 $\pm$ 0.68	0.29	25.01 $\pm$ 17.59	0.10	0.15 $\pm$ 0.21	0.15
Male	279	3.19 $\pm$ 0.70		1.95 $\pm$ 0.61		2.19 $\pm$ 0.70		22.37 $\pm$ 17.59		0.12 $\pm$ 0.21	
Height-for-age (Z-score)											
-1 to < 0	186	3.28 $\pm$ 0.70	0.06	1.97 $\pm$ 0.60	0.02*	2.28 $\pm$ 0.70	0.06	23.28 $\pm$ 16.74	0.54	0.12 $\pm$ 0.21	0.23
0 to 1	206	3.21 $\pm$ 0.72		1.92 $\pm$ 0.65		2.21 $\pm$ 0.72		23.98 $\pm$ 21.22		0.12 $\pm$ 0.20	
> 1	152	3.16 $\pm$ 0.64		1.85 $\pm$ 0.58		2.16 $\pm$ 0.64		23.92 $\pm$ 18.01		0.16 $\pm$ 0.22	
BMI-for-age (Z-score)											
< -2	81	3.31 $\pm$ 0.77	0.02*	2.05 $\pm$ 0.71	0.13	2.31 $\pm$ 0.77	0.02*	24.81 $\pm$ 22.64	0.002*	0.13 $\pm$ 0.20	<0.0001*
-2 to 1	306	3.16 $\pm$ 0.67		1.86 $\pm$ 0.59		2.21 $\pm$ 0.67		21.28 $\pm$ 14.90		0.10 $\pm$ 0.20	
> 1 to 2	97	3.25 $\pm$ 0.67		1.94 $\pm$ 0.59		2.25 $\pm$ 0.67		26.33 $\pm$ 22.43		0.17 $\pm$ 0.21	
> 2	60	3.37 $\pm$ 0.68		2.00 $\pm$ 0.61		2.37 $\pm$ 0.68		30.49 $\pm$ 22.84		0.26 $\pm$ 0.22	
% Body fat (terciles)											
$\leq$ 15.7	178	3.20 $\pm$ 0.75	0.11	1.91 $\pm$ 0.68	0.37	2.20 $\pm$ 0.75	0.11	21.58 $\pm$ 19.36	0.001*	0.11 $\pm$ 0.20	<0.0001*
15.7 to 24.4	175	3.15 $\pm$ 0.63		1.86 $\pm$ 0.57		2.15 $\pm$ 0.63		21.38 $\pm$ 14.10		0.09 $\pm$ 0.19	
> 24.4	191	3.31 $\pm$ 0.67		1.97 $\pm$ 0.58		2.31 $\pm$ 0.67		27.89 $\pm$ 21.44		0.20 $\pm$ 0.22	
Screen time (hours/day) ¹											
$\leq$ 2	241	3.22 $\pm$ 0.67	0.83	1.92 $\pm$ 0.60	0.80	2.22 $\pm$ 0.67	0.83	23.18 $\pm$ 18.14	0.37	0.13 $\pm$ 0.21	0.98
> 2	303	3.22 $\pm$ 0.71		1.91 $\pm$ 0.63		2.22 $\pm$ 0.71		24.16 $\pm$ 19.44		0.13 $\pm$ 0.22	
Per capita income (US\$) [†]											
< 90.1 (1° tercile)	182	3.30 $\pm$ 0.75	0.82	1.97 $\pm$ 0.66	0.35	2.30 $\pm$ 0.75	0.82	22.75 $\pm$ 19.73	0.03*	0.11 $\pm$ 0.21	0.01*
90.1 to 157.8 (2° tercile)	181	3.22 $\pm$ 0.69		1.90 $\pm$ 0.62		2.22 $\pm$ 0.69		23.84 $\pm$ 19.23		0.15 $\pm$ 0.20	
> 157.8 (3° tercile)	181	3.15 $\pm$ 0.63		1.89 $\pm$ 0.57		2.15 $\pm$ 0.63		24.49 $\pm$ 17.70		0.14 $\pm$ 0.21	
Maternal education (years)											
$\leq$ 4	66	3.11 $\pm$ 0.52	0.01*	1.75 $\pm$ 0.46	<0.0001*	2.11 $\pm$ 0.52	0.01*	19.38 $\pm$ 12.83	0.003*	0.11 $\pm$ 0.22	0.01*
5 to 10	168	3.18 $\pm$ 0.69		1.86 $\pm$ 0.62		2.18 $\pm$ 0.69		22.46 $\pm$ 20.90		0.12 $\pm$ 0.21	
11	210	3.27 $\pm$ 0.72		1.98 $\pm$ 0.64		2.27 $\pm$ 0.72		23.98 $\pm$ 16.79		0.14 $\pm$ 0.21	
$\geq$ 12	98	3.29 $\pm$ 0.71		1.99 $\pm$ 0.64		2.29 $\pm$ 0.71		28.59 $\pm$ 21.92		0.17 $\pm$ 0.19	

Abbreviations: SD: Standard Deviation; BMI: body mass index; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; TC: Total cholesterol; AC: atherosclerosis coefficient; LCI: lipoprotein combined index; AIP: atherogenic index of plasma.

Linear regression models with lipid markers and, atherogenic indexes as the continuous outcome. For variables with three or more categories, P-value was from Wald test for linear trend representing ordinal categories as continuous. Robust variance estimations were specified in all models.

[†]Approximate exchange rates of real (R\$) to dollar (US\$) at the time of this study (US\$1.00 = R\$ 3.33). * $P < 0.05$

Table 2. Association of the risk allele of the *FTO* rs9939609 with the lipid markers and atherogenic indexes in children from Viçosa, Minas Gerais, Brazil, 2015.

Markers	Unadjusted difference (95% CI) ¹			Adjusted difference (95% CI) ²		
	<i>FTO</i> gene			<i>FTO</i> gene		
	TT	AT/AA	<i>P</i> value	TT	AT/AA	<i>P</i> value
Total cholesterol (mg/dL)	Reference	0.75 (-4.02, 5.53)	0.76	Reference	0.04 (-4.76, 4.85)	0.99
Triglyceride (mg/dL)	Reference	-4.14 (-10.35, 2.07)	0.19	Reference	-3.32 (-9.19, 2.54)	0.27
HDL-c (mg/dL)	Reference	-0.64 (-2.56, 1.28)	0.51	Reference	-1.01 (-2.94, 0.91)	0.30
LDL-c (mg/dL)	Reference	1.29 (-2.81, 5.40)	0.54	Reference	0.90 (-3.19, 5.01)	0.66
Non-HDL-c (mg/dL)	Reference	1.09 (-3.25, 5.45)	0.62	Reference	0.65 (-3.73, 5.04)	0.77
TC/HDL-c	Reference	0.05 (-0.06, 0.18)	0.35	Reference	0.06 (-0.05, 0.18)	0.27
LDL-c/HDL-c	Reference	0.05 (-0.05, 0.16)	0.35	Reference	0.05 (-0.05, 0.16)	0.29
AC	Reference	0.05 (-0.06, 0.18)	0.34	Reference	0.06 (-0.05, 0.19)	0.27
LCI	Reference	-0.23 (-3.69, 3.21)	0.89	Reference	0.15 (-3.12, 3.42)	0.93
AIP	Reference	0.00 (-0.05, 0.03)	0.63	Reference	0.00 (-0.04, 0.03)	0.95

Abbreviations: 95%CI: 95% Confidence Interval; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; TG: Triglyceride; AC: atherosclerosis coefficient; LCI: lipoprotein combined index; AIP: atherogenic index of plasma; TT: individuals without polymorphism; AT: heterozygous individuals with a risk allele; AA: individuals with polymorphism in the *FTO* gene.

¹ From linear regression models with each lipid marker as a continuous outcome and *FTO* gene categories as predictor. Robust estimates of variance were specified in all models.

² From linear regression adjusted for sex, age, screen time, per capita income, maternal education, total energy intake and % body fat.

Table 3. Association of the risk allele of the *MC4R* rs17782313 with the lipid markers and atherogenic indexes in children from Viçosa, Minas Gerais, Brazil, 2015.

Markers	Unadjusted difference (95% CI) ¹			Adjusted difference (95% CI) ²		
	<i>MC4R</i> gene			<i>MC4R</i> gene		
	TT	TC/CC	<i>P</i> value	TT	TC/CC	<i>P</i> value
Total cholesterol (mg/dL)	Reference	0.51 (-4.09, 5.11)	0.83	Reference	1.68 (-3.11, 6.48)	0.49
Triglyceride (mg/dL)	Reference	-0.32 (-5.96, 5.32)	0.91	Reference	0.57 (-5.08, 6.23)	0.84
HDL-c (mg/dL)	Reference	0.89 (-1.01, 2.79)	0.36	Reference	0.83 (-1.10, 2.77)	0.40
LDL-c (mg/dL)	Reference	-1.37 (-5.42, 2.67)	0.51	Reference	0.03 (-4.09, 4.17)	0.99
Non-HDL-c (mg/dL)	Reference	-0.05 (-4.36, 4.24)	0.98	Reference	1.05 (-3.44, 5.55)	0.65
TC/HDL-c	Reference	0.00 (-0.12, 0.12)	0.99	Reference	0.02 (-0.10, 0.16)	0.67
LDL-c/HDL-c	Reference	-0.02 (-0.13, 0.08)	0.70	Reference	0.01 (-0.10, 0.12)	0.85
AC	Reference	0.00 (-0.12, 0.12)	0.99	Reference	0.02 (-0.10, 0.16)	0.67
LCI	Reference	0.53 (-2.88, 3.95)	0.76	Reference	1.56 (-2.12, 5.25)	0.41
AIP	Reference	-0.01 (-0.04, 0.02)	0.66	Reference	0.00 (-0.04, 0.03)	0.78

Abbreviations: 95%CI: 95% Confidence Interval; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; TG: Triglyceride; AC: atherosclerosis coefficient; LCI: lipoprotein combined index; AIP: atherogenic index of plasma, TT: individuals without polymorphism; TC: heterozygous individuals with a risk allele; CC: individuals with polymorphism in the *MC4R* gene.

¹From linear regression models with each lipid marker as a continuous outcome and *MC4R* gene categories as predictor. Robust estimates of variance were specified in all models.

²From linear regression adjusted for sex, age, screen time, per capita income, maternal education, total energy intake and % body fat.

Table 4. Association of the risk allele of the *MTMR9* rs2293855 with the lipid markers and atherogenic indexes in children from Viçosa, Minas Gerais, Brazil, 2015.

Markers	Unadjusted difference (95% CI) ¹			Adjusted difference (95% CI) ²		
	<i>MTMR9</i> gene			<i>MTMR9</i> gene		
	GG	AG/AA	<i>P</i> value	GG	AG/AA	<i>P</i> value
Total cholesterol (mg/dL)	Reference	1.21 (-3.63, 6.05)	0.62	Reference	1.33 (3.60, 6.27)	0.60
Triglyceride (mg/dL)	Reference	1.57 (-3.68, 6.83)	0.56	Reference	2.53 (-2.72, 7.80)	0.34
HDL-c (mg/dL)	Reference	-1.41 (-2.74, -0.08)	0.04*	Reference	-1.75 (-3.12, -0.38)	0.01*
LDL-c (mg/dL)	Reference	2.40 (-2.04, 6.85)	0.29	Reference	2.76 (-1.69, 7.22)	0.22
Non-HDL-c (mg/dL)	Reference	2.74 (-2.13, 7.62)	0.27	Reference	3.12 (-1.77, 8.02)	0.21
TC/HDL-c	Reference	0.12 (-0.01, 0.26)	0.08	Reference	0.15 (0.01, 0.29)	0.03*
LDL-c/HDL-c	Reference	0.11 (-0.01, 0.23)	0.08	Reference	0.13 (0.01, 0.25)	0.03*
AC	Reference	0.12 (-0.01, 0.26)	0.08	Reference	0.15 (0.01, 0.29)	0.03*
LCI	Reference	3.60 (-0.66, 7.87)	0.10	Reference	4.21 (-0.01, 8.57)	0.05
AIP	Reference	0.02 (-0.01, 0.05)	0.19	Reference	0.02 (-0.01, 0.05)	0.10

Abbreviations: 95%CI: 95% Confidence Interval; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; TG: Triglyceride; AC: atherosclerosis coefficient; LCI: lipoprotein combined index; AIP: atherogenic index of plasma; GG: individuals without polymorphism; AG: heterozygous individuals with a risk allele; AA: individuals with polymorphism in the *MTMR9* gene.

¹From linear regression models with each lipid marker as a continuous outcome and *MTMR9* gene categories as predictor. Robust estimates of variance were specified in all models.

²From linear regression adjusted for sex, age, screen time, per capita income, maternal education, total energy intake and % body fat.

**P* value <0.05

Table 5. Association of the risk allele of the genetic risk score with the lipid markers and atherogenic indexes in children from Viçosa, Minas Gerais, Brazil, 2015.

Markers	Unadjusted difference (95% CI) ¹			Adjusted difference (95% CI) ²		
	Genetic risk score			Genetic risk score		
	0	≥1	<i>P</i> value	0	≥1	<i>P</i> value
Total cholesterol (mg/dL)	Reference	-0.90 (-6.25, 4.44)	0.74	Reference	-0.07 (-5.42, 5.26)	0.98
Triglyceride (mg/dL)	Reference	3.18 (-2.99, 9.36)	0.31	Reference	4.50 (-1.50, 10.52)	0.14
HDL-c (mg/dL)	Reference	-2.10 (-4.06, -0.14)	0.04*	Reference	-2.11 (-4.08, -0.14)	0.04*
LDL-c (mg/dL)	Reference	-0.45 (-5.23, 4.31)	0.85	Reference	0.29 (-4.33, 4.92)	0.90
Non-HDL-c (mg/dL)	Reference	1.47 (-3.67, 6.62)	0.58	Reference	2.20 (-2.85, 7.27)	0.39
TC/HDL-c	Reference	0.15 (0.001, 0.29)	0.04*	Reference	0.16 (0.02, 0.31)	0.02*
LDL-c/HDL-c	Reference	0.09 (-0.03, 0.23)	0.13	Reference	0.11 (-0.01, 0.24)	0.07
AC	Reference	0.15 (0.002, 0.29)	0.04*	Reference	0.16 (0.02, 0.31)	0.02*
LCI	Reference	3.61 (-0.43, 7.65)	0.08	Reference	4.36 (0.3, 8.36)	0.03*
AIP	Reference	0.03 (0.00, 0.07)	0.07	Reference	0.04 (0.004, 0.08)	0.03*

Abbreviations: 95%CI: 95% Confidence Interval; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; TG: Triglyceride; AC: atherosclerosis coefficient; LCI: lipoprotein combined index; AIP: atherogenic index of plasma.

¹From linear regression models with each lipid marker as a continuous outcome and genetic score categories as predictor. Robust estimates of variance were specified in all models.

²From linear regression adjusted for sex, age, screen time, per capita income, maternal education, total energy intake and % body fat.

**P* value <0.05