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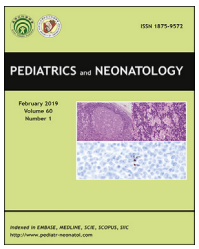

Original Article

The impact of TNF-α 308G > A gene polymorphism on children's overweight risk and an assessment of biochemical variables: A cross-sectional single-center experience

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Key Words
children;
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Introduction: The aim of this study was to assess the role of *TNF-α 308 G>A* gene polymorphism in children's *overweight risk* so as to correlate this polymorphism with *anthropometric and biochemical variables*.
Materials and method: A cross-sectional study was carried out on 188 Romanian children ages 5–18 years, who were classified into controls (Group 1; n = 109) and overweight children (Group 2; n = 79).
Results: Higher values of MUAC and TST ($p < 0.001$) were obtained in the overweight group. A significant association was found between *TNF-α 308 G>A polymorphism* and weight status in

Abbreviation: A/A, homozygous for the A allele; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BMI, body mass index; Chol, total cholesterol; CI, confidence interval; G/A, heterozygous; G/G, homozygous for the G allele; Gly, glycemia; H/L, height/length; HDL-chol, high-density lipoprotein cholesterol; IL, interleukin; INF, interferon; IOFT, International Obesity Task Force; LDL-chol, low-density lipoprotein cholesterol; MUAC, mid-upper arm circumference; N, absolute number; OR, odds ratio; TG, triglycerides; TNF, tumor necrosis factor; TST, tricipital skinfold thickness; W, weight.

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the studied population ($p = 0.009$). There was also a positive association between the variant genotypes (GA or AA) of *TNF-α 308G>A* gene polymorphism and weight status, which was more frequently found among normal weight than overweight children (74.5% versus 25.5%, respectively). The final logistic multivariable included five independent variables (*TNF-α* genotype, gender, cholesterol, ASAT, and ALAT), which were statistically significant predictors with negative/positive effects on children's overweight risk; this model explained 30% of the variance in the outcome variable.

Conclusion: The variant genotype of *TNF-α 308G>A* gene polymorphism was more frequent among normal weight children. In the presence of other covariates, such as age, gender, cholesterol, LDL cholesterol, ALAT, and glycemia, the *TNF-α 308 G>A* gene polymorphism remained an independent protective factor for children's overweight status.

1. Introduction

The prevalence of obesity in both children and adults has increased dramatically in recent years, with the condition now regarded as an epidemic phenomenon.[1] According to the Centers for Disease Control and Prevention, the highest incidence of obesity was reported in the United States,[2] estimated at over 40% in 2010. This is similar to the 38% incidence rate reported in Europe, compared with only 22% in Southeast Asia.[3] In Romania, the incidence of obesity varies from 5.75% to 29% in different parts of the country, whereas the prevalence of overweight ranges between 7% and 18.2%.[4,5]

There are multiple factors that contribute to children's nutritional disorders. Different studies have proven that the impact of diet on obese genotype patients is partially determined by genetic factors.

Pro-inflammatory cytokines are believed to play a role in the pathogenesis of nutritional disorders. Thus, they have recently been increasingly studied, with published reports indicating that the level of inflammatory molecules is usually increased in obesity.[6]

In obese patients, the adipose tissue is characterized by a decreased degree of inflammation and an increased level of certain cytokines.[7] Studies have reported a positive correlation between tumor necrosis factor (*TNF*)-α, interleukin (IL)-6, leptin level, adipose mass, and body mass index (BMI).[6,8] Among these, TNF-α and IL-6 have been found to present the worst outcome because they alter the normal function of adipose tissue, influencing adipogenesis and contributing to complications of obesity.[7]

TNF-α is an important modulator of gene expression that is correlated not only with obesity itself but also with obesity-causing disorders. *TNF-α* affects lipid metabolism and can lead to hypertriglyceridemia by decreasing lipase activity and increasing *de novo* fatty acid synthesis in the liver.[9] *TNF-α 308 G>A* influences gene expression by increasing the expression of this cytokine in adipose tissue (a modulator of this gene). The A allele of this gene is more frequent in obese children; therefore, it is the most studied gene polymorphism.[9,10]

Arner, in a study on premenopausal women, reported a positive relationship between *TNF-α* secretion and BMI, total body fat, and adipocyte volume, with the levels of *TNF-α* being increased in patients with adipose hypertrophy and decreased in those with adipose hyperplasia. Serum *TNF-α* in lean premenopausal women was found to present an important role in determining the overall adipose tissue mass and volume by regulating adipogenesis or depositing lipids in adipocytes.[7]

TNF-α expression can be modulated by the obesity degree and/or insulin plasma levels. On this basis, other studies have tried to establish a connection between *TNF-α* gene polymorphisms and obesity or degree of overweight. For instance, Chang proved that *TNF-α 308 G/A* polymorphism is associated with obesity.[11]

Based on the above-mentioned hypotheses, by using BMI as a relevant indicator of nutritional status, the present research aimed: i) to determine whether *TNF-α 308G>A gene polymorphism is associated with overweight status*, ii) to determine whether *TNF-α 308G>A gene polymorphism is related to anthropometric (body mass index (BMI), mid-upper arm circumference (MUAC), and tricipital skinfold thickness (TST)) and biochemical (proteins, cholesterol, triglycerides, and lipids) variables*, and iii) to *quantify the influence of the studied gene polymorphism on overweight risk in children* by assessing certain laboratory variables (cholesterol, LDL cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and glycemia (Gly).

2. Materials and method

2.1. Subjects

A cross-sectional study was carried out on 188 Romanian children ages 5—18 years. The subjects were evaluated in a pediatric tertiary hospital in Romania from January 2012 to January 2017. The children were divided into two groups according to age- and gender-specific body mass index (BMI kg/m^2): Group 1 included 109 controls, and Group 2 consisted of 79 overweight children.

The control group included healthy children with normal weight (BMI z score > -2 SD and BMI z score ≤ 1.0 SD) and without any underlying diseases. The overweight

group included overweight pediatric patients (BMI z score between 1.0 SD and 2.0 SD),[12,13] also without underlying conditions. The exclusion criteria for the control group were: patients with prior diagnosis of any chronic disorders, subjects who followed a restrictive diet, and children whose parents refused to sign the informed consent. For the overweight group, the exclusion criteria were: subjects with other diagnosed medical conditions, children who presented other complaints besides overweight, patients diagnosed with secondary hypercholesterolemia, and subjects whose parents did not sign the informed consent. According to the World Health Organization (WHO)[14] and the International Obesity Task Force (IOTF),[15] overweight was defined as a BMI between + 1.0 and 2.0 SD.

The subjects' parents gave their written informed consent for the inclusion of their children in the study, which was carried out in compliance with the principles of the Helsinki Declaration and was approved by the Ethics Committee of the University of Medicine and Pharmacy of (No. 13/July 2011).

2.2. Outcome variable

Body mass index (BMI) was used as the response variable in the analysis. This was calculated by dividing the weight (kg) by the standing height squared (m^2). According to data from the Centers for Disease Control and Prevention (reference), the subjects were classified into two groups: normal BMI (z-score from −2 to 1 SD) and overweight (BMI z-score > 1 SD).

2.3. Exposure variables

2.3.1. Anthropometric measurements

The anthropometric variables, such as weight (kg), height (cm), MUAC, and TST, were measured by a single trained person. The body weight was determined by using a daily calibrated scale with ±10-g error. The height was measured with the use of a daily calibrated pedometer evaluated in SD (0.1-cm error).

The MUAC was obtained at the midpoint between the shoulder tip and the elbow by using a tape measure calibrated in centimeters, whereas the TST was measured in the posterior upper arm by using a thickness caliper. The values of these anthropometric variables were converted to SD for age and sex according to the Switzerland Growth Chart—growth curves with the use of the Growth Analyzer software version 3.5;[16] the physiologic reference range was between −2.0 and + 2.0 SD.

2.3.2. Laboratory variables

For all the surveyed cases, the following laboratory tests were carried out to determine the levels of cholesterol, LDL cholesterol, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and glycemia (Gly). The cholesterol and LDL-chol levels were measured by spectrophotometry on a Cobas Integra 400 Plus automated analyzer and were considered normal at levels <170 mg/dl; the percentiles for age and gender were also reported according to the standard table values.[17]

2.3.3. Genotyping description

Rapid isolation of total DNA was done from 100 μl of fresh blood samples by using the Quick-gDNA MiniPrep kit (ZymoResearch) according to the manufacturer's instructions.

TNF-α 308G>A polymorphism genotyping was carried out by using the amplification refractory mutation system—polymerase chain reaction (ARMS-PCR) assay with primers (Eurogentec) previously described by Daneshmandi et al.[18] but with modifications to the PCR protocol.

The PCR consisted of an initial denaturation of 7 min at 95 °C, followed by 35 cycles at 94 °C for 30s, at 57 °C for 30s, and at 72 °C for 40s, with a final extension of 7 min at 72 °C. The PCR products were separated by applying 2% agarose gel electrophoresis (TopVision Agarose; Thermo Scientific) with ethidium bromide staining.

2.4. Statistical analysis

The advanced statistical environment R (version 3.2.4; Vienna, Austria) was used in the entire statistical analysis. To assess the normality of continuous variables (e.g., BMI, MUAC, and TST), the Kolmogorov—Smirnov and Shapiro—Wilk tests were applied. Student's *t* test was used to evaluate the differences between the means of continuous variables (expressed as means ± SD), whereas the differences between nonparametric variables (expressed as medians and ranges) were compared by using the Mann—Whitney test. The differences among biochemical variables and the three genotype groups of the *TNF-α 308 G>A* gene polymorphism (i.e., G/G, G/A, and A/A) were estimated by using the Kruskal—Wallis test, which is appropriate for the analysis of more than two groups. To assess the associations between the studied genotype distribution and other laboratory and anthropometric variables, contingency tables and the chi-square test were used. The possible involvement of *TNF-α 308 G>A* gene polymorphism in overweight risk was tested by logistic regression, and the magnitude of association was estimated by the adjusted odds ratio (OR) to show the probability of overweight status according to the given polymorphism while controlling for anthropometric and biochemical variables. For all bilateral tests, statistical significance was set at p-values lower than the significance threshold ($\alpha = 0.05$).

3. Results

3.1. Sample characteristics

The children were grouped according to their BMI z-score, with Group 1 consisting of 109 healthy control subjects, and Group 2 comprising 79 overweight children. The Hardy—Weinberg equilibrium was calculated, with the following results: χ^2 (1) = 0.28 and p = 0.597 for the control group, and χ^2 (1) = 14.44 and p = 0.0001 for the study group.

The mean ± SD age in the two groups was similar: 11.00 ± 3.70 years in the control group, and 10.10 ± 3.20 years in the overweight group; there was no significant difference between them (p = 0.073). Also, the two groups had a similar gender distribution (p = 0.228).

3.2. Distribution of anthropometric and biochemical values

The *t* test showed a significant difference in the distributions of the z-scores for anthropometric variables, such as MUAC and TST ($p < 0.001$), which were much higher in the overweight group (Table 1). Also, there were significant differences in cholesterol, LDL cholesterol, GPT, and glycemic levels between normal and overweight children (Table 1).

The serum *TNF-α* level was determined only for 23 control patients and 58 overweight children, whereas IL-6 was assessed in 11 controls and 37 overweight children. These variables could not be assessed in all the children in the study due to limitations in both the laboratory conditions and the sample collection; however, the relationship of these variables with *TNF-α 308G>A* gene polymorphism and their role in the risk of developing overweight in children are worth emphasizing.

The Mann–Whitney test showed significant differences in *TNF-α* concentrations between the two groups in this study ($p = 0.042$), with the overweight children presenting significantly higher values than the controls (Table 1). The same test also found differences in IL-6 concentrations between normal weight and overweight children ($p = 0.008$); the latter had significantly higher IL-6 levels compared with the control group (Table 1).

3.3. Association between TNF-α 308G > A polymorphism and weight status

The chi-square test showed an association between the variant genotype (GA or AA) of the *TNF-α 308G>A gene polymorphism* and weight status (χ^2 (1) = 8.76; $p = 0.003$), with the variant genotype being more frequent among normal weight than overweight children (74.5% versus 25.5%, respectively). The studied genotypes of this gene polymorphism were in Hardy–Weinberg equilibrium in the population ($p > 0.05$).

3.4. Association between TNF-α 308G > A polymorphism and the laboratory variables

To determine the association between *TNF-α 308G>A gene polymorphism* and the biochemical variables, the distributions of the studied variables were compared among the carriers of each genotype (Table 2). The Kruskal–Wallis test found no significant difference in any of the studied variables ($p > 0.05$).

3.5. Influence of TNF-α 308 G > A gene polymorphism on overweight risk

Logistic regression analysis was carried out to determine the effect of *TNF-α 308 G>A* gene polymorphism on overweight risk; the effect was adjusted for the presence of several biochemical variables (Table 3). The tested model was found to be statistically significant (χ^2 (8) = 36.45; $p < 0.001$), with the Hosmer–Lemeshow statistic (χ^2_{HL} (8) = 13.34; $p = 0.101$) indicating a reasonable fit between the proposed model and the data. The model accounted for 30% (Nagelkerke R^2 coefficient) of the variance in the outcome variable and correctly classified 59% of the cases (an overall classification of 72.5%). The results of the logistic regression analysis (Table 3) highlighted five

Table 1 Description of studied groups regarding anthropometric and laboratory parameters.

Variables	Weight status		p-value
	Normal, $n_1 = 109$	Overweight, $n_2 = 79$	
Age (years)[a]	11.00 ± 3.70	10.10 ± 3.20	0.073
Gender M/F[b]	51(46.8)/58(53.2)	44(55.7)/35 (44.3)	0.228
BMI z scores[a]	−0.32 ± 0.80	2.52 ± 0.88	<0.001
Height z scores[c]	−0.35 [−1.32; 0.51]	0.78 [−0.05; 1.87]	<0.001
Weight z scores[c]	−0.33 [−1.08; 0.48]	3.09 [2.39; 3.81]	<0.001
MUAC z-score[a]	−0.421 ± 2.41	6.28 ± 4.68	<0.001
TST z-score[a]	0.33 ± 6.70	3.56 ± 2.70	<0.001
Chol (mg/dl)[c]	154.50 [132.00; 172.50]	173.30 [143.50; 186.00]	0.001
LDL-chol (mg/dl)[c]	85.00 [69.00; 100.00]	93.00 [77.00; 116.00]	0.034
HDL-chol (mg/dl)[c]	48.00 [37.90; 61.60]	48.00 [41.20; 60.40]	0.840
TG (mg/dl)[c]	64.00 [50.80; 83.80]	82.85 [56.03; 138.50]	0.002
ASAT (U/L)[c]	25.00 [19.95; 31.00]	24.90 [21.30; 31.00]	0.186
ALAT (U/L)[c]	14.50 [11.00; 18.50]	23.00 [16.20; 33.60]	<0.001
Gly (mg/dl)[c]	84.00 [77.00; 89.50]	87.00 [80.80; 95.90]	0.036
TNF-α (pg/ml)[c,d]	35.74 [19.45; 83.53]	59.70 [33.69; 260.50]	0.042
IL-6 (pg/ml)[c,e]	6.10 [0.81; 54.80]	122.90 [20.20; 191.12]	0.008

ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; Chol = total cholesterol; Gly = glycemia; LDL-chol = low density lipoprotein cholesterol; MUAC = middle upper arm circumference; n = absolute number; p = values from Student-t or Mann–Whitney test; TST = tricipital skinfold thickness.

[a] Arithmetic mean ± SD = standard deviation.

[b] Absolute (relative) frequency; [(b] absolute (relative %) frequency.

[c] Median [percentile 25%, percentile 75%].

[d] $n_1 = 23$, $n_2 = 58$.

[e] $n_1 = 11$, $n_2 = 37$.

Table 2 Description of the association between *TNF-α 308G>A* gene polymorphism and laboratory parameters stratified by studied groups.

TNF-α 308G>A genotypes	Normal weight			p-value	Overweight			p-value
	GG (n_1)	GA (n_2)	AA (n_3)		GG (n_4)	GA (n_5)	AA (n_6)	
Chol (mg/dl)[a]	150.75 [132.00; 174.95]	153.00 [124.35; 163.15]	172.00 [155.00; 195.00]	0.137	174.15 [144.60; 189.85]	163.80 [135.00; 181.80]	153.50 [131.60; 186.00]	0.683
LDL (mg/dl)[a]	85.00 [71.75; 113.50]	77.80 [55.00; 93.00]	96.65 [93.00; 115.50]	0.125	92.00 [77.00; 116.00]	93.00 [82.70; 118.00]	77.00 [70.00; 90.00]	0.405
ASAT (U/L)[a]	25.65 [19.00; 31.00]	22.85 [19.70; 27.10]	26.50 [17.00; 42.00]	0.510	24.10 [21.30; 29.00]	23.00 [21.70; 29.10]	35.50 [31.45; 38.90]	0.090
ALAT (U/L)[a]	14.60 [12.00; 18.90]	13.00 [10.20; 17.00]	18.00 [9.00; 27.00]	0.567	21.00 [16.00; 29.00]	27.50 [22.00; 36.00]	34.10 [28.50; 37.75]	0.136
Gly (mg/dl)[a]	84.50 [78.00; 90.20]	85.15 [76.00; 89.50]	86.20 [83.40; 90.00]	0.886	85.50 [80.70; 95.90]	89.90 [81.60; 98.00]	88.00 [82.00; 89.50]	0.716

AA — homozygous for A allele, ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; Chol = total cholesterol; Gly = glycemia; GA - heterozygous, GG — homozygous for G allele, LDL-chol = low density lipoprotein cholesterol; n_i = number of cases where n_1 between 54 and 66; n_2 between 30 and 35; n_3 = 6; n4 between 57 and 64; n_5 between 7 and 10; n_6 between 3 and 5).

[a] Data were described as median and IQR = interquartile range [percentile 25%; percentile75%].

independent significant predictors of children's overweight risk, with the *TNF-α* variant genotype shown to have a negative effect on overweight risk; i.e., it is a protective factor for overweight status (OR = 0.34, 95%CI: 0.13—0.87).

4. Discussion

TNF-α 308 G>A gene polymorphism is a major metabolism regulator and has been reported to be involved in metabolic disorders in both adults and children. Although some genetic studies on *TNF-α 308 G>A* gene polymorphism in adult populations have been carried out, only a few works have focused on metabolic disorders, and only some of these have underlined the potential role of the *TNF-α* gene in children's nutritional disorders.[10,19] There are very limited data on the involvement of *TNF-α 308 G>A* gene polymorphism in adiposity and inflammatory status.[10] Obesity is well known to be an inflammatory status; however, *TNF-α* has also been reported to be involved in other inflammatory conditions in children, such as gastritis.[20]

Nevertheless, multiple *TNF-α* gene polymorphisms have been found to be involved in nutritional disorders in children and adults; including *TNF-α 308 G>A, TNF-α 238 G>A*.[8,21] Among these gene polymorphisms, only *TNF-α 308 G>A* gene polymorphism has been associated with obesity. Thus, based on the above-mentioned facts, the present study focuses on *TNF-α 308 G>A* gene polymorphism.

Among the works that have studied the relationship between *TNF-α* levels and patient age and BMI, one research[8] underlined that the G/G genotype was related to higher *TNF-α* levels in males with normal weight, without any notable effect in males with a BMI of over 25 kg/m^2. Regarding age, the same study observed a continuous decrease in *TNF-α* levels between 5 and 17 years,[8] whereas Sack et al.[22] in 1998 showed an increase in *TNF-α* levels in the age group of 5—10 years, followed by a decrease in the age group of 10—15 years. The literature includes some studies that have assessed the relationship between the alleles of *TNF-α 308 G>A* gene polymorphism and obesity status, such as Sack et al.,[22] Ziccardi et al.,[23] Fernandez-Real et al.,[24] and Dalziel et al.;[25] Table 4 presents a comparison of the findings of these works. Sack et al.[22] noted that despite their observation of increased *TNF-α* levels in overweight *A*-allele carriers, obesity alone was not enough to produce a significant modification between the *TNF-α 308* genotype and *TNF-α* serum levels. Similarly, the present study found that the serum levels of *TNF-α* and *IL-6* were higher in overweight children compared with the control group. On the other hand, some of our previous studies showed that obesity in children could also be related to the presence of the C allele of *IL-6174 G/C* and *IL-6 C/T* gene polymorphisms[26] or of *LEPR* 223 and *LEPR* 1019 gene polymorphisms,[27] or with the D allele of ACE I/D gene polymorphism.[28] Other studies have reported that obesity in children seems to be related also to the genetic features of the mother. Therefore, it was emphasized that mothers carrying a variant allele of *TGF-β1 869 T>C* polymorphism and the C allele of the *PPARγ2* 34 C>*G* gene polymorphism have a higher risk for obesity in their

Table 3 The influence of *TNF-α 308G>A* gene polymorphism on overweight risk.

Predictors	Univariable regression analysis		Multivariable regression analysis	
	p-value	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)
TNF-α (GA genotype vs. GG)	0.003	0.30 [0.14; 0.66]	Not included in multivariable model[a]	
TNF-α (AA genotype vs. GG)	0.847	0.89 [0.26; 3.04]	Not included in multivariable model[a]	
TNF-α (GA + AA genotype vs. GG)	0.004	0.36 [0.18; 0.72]	0.024	0.34 [0.13; 0.87]
Age (years)	0.074	0.93 [0.85; 1.01]	0.356	0.95 [0.84; 1.06]
Gender (Male vs. Female)	0.056	1.77 [0.99; 3.18]	0.044	2.24 [1.02; 4.91]
Chol (mg/dl)	0.003	1.01 [1.01; 1.02]	0.002	1.02 [1.01; 1.03]
ASAT (U/L)	0.129	1.02 [0.99; 1.04]	0.026	0.94 [0.89; 0.99]
ALAT (U/L)	0.001	1.04 [1.01; 1.07]	0.001	1.07 [1.03; 1.11]
Gly (mg/dl)	0.739	1.00 [0.99; 1.01]	0.849	1.00 [0.99; 1.01]

AA — homozygous for A allele, the predominant genotype was G/G (70.7%; 68 normal weight children and 65 overweight), the heterozygous genotype G/A was identified in 34 normal weight and 10 overweight children while the homozygous genotype A/A was present in 5.9% of the studied population (7 normal weight and 4 overweight children); ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; Chol = total cholesterol; CI = confidence interval; Gly = glycemia; GA - heterozygous, GG - homozygous for G allele, LDL-chol = low density lipoprotein cholesterol; n = absolute number; OR = odds ratio; p = values, TNF — tumor necrosis factor.

[a] Small number of events (number overweight patients) in relation to the number of predictors.

children,[29] whereas the presence of the variant genotype of *IL-6 -572 C>G* gene polymorphism in mothers is a protective factor for newborn obesity risk.[30] Similarly, both *FTO* rs9939609 and *LEPR* rs1137101 gene polymorphisms also have an important role in the neonatal weight status, as predictors of potential obesity development.[31,32]

Ziccardi et al.[23] also assessed the relationship between secretion of IL-6, in addition to *TNF-α*, and obesity, with the conclusion that obese patients present an inadequate secretion of IL-6. In the present study, no significant difference in age or gender was found between the control and overweight groups, most probably due to the relatively small number of cases included in the research; however, our main goal is to extend our investigation in the future.

In contrast to the findings of Fernandez-Real et al.[24] and Dalziel et al.,[25] the current work found a significant association between the variant genotype (GA or AA) of *TNF-α 308 G>A gene polymorphism* and weight status ($p = 0.003$), which was more frequent among normal weight than overweight children (74.5% versus 25.5%, respectively). Dalziel et al. also noted that obese carriers of the *TNF-α 308 A* allele had a net association between insulin resistance and HDL cholesterol, which suggested the increased effect of dyslipidemia in developing insulin resistance in these patients.[25] However, no statistically significant association between *TNF-α 308G>A gene polymorphism* and any of the studied biochemical variables was found in the present work ($p > 0.05$).

Brand et al. reported results similar to those in the current research. They found that the *G* allele of *TNF-α 308 G>A* was associated with BMI, especially in the highest BMI quartile (>36.5 kg/m^2); however, they did not find any

Table 4 The relation between *TNF-α 308 G>A* gene polymorphism and obesity, revealed in the literature.

No.	Value of serum level of *TNF-α*	Age (years)	Variant allele of *TNF-α 308 G>A* gene polymorphism	Study
1.	Increased values decreased values	5—10 years 10—15 years	A allele in overweight children	Sack et al.[22]
2.	Inadequate secretion of *TNF-α*	25—44 years 56 obese women (35.3 ± 4.8)/40 non-obese (34.1 ± 5.2)	The authors did not evaluate the *TNF-α 308 G>A gene polymorphism*	Ziccardi et al.[23]
3.	Association between serum *TNF-α and gene polymorphism of TNF-α*	19 men (36.2 ± 1.9 years) and 19 women (34.9 ± 1.4 years)	A allele in obese subjects	Fernandez-Real[24]
4.	Association between serum *TNF-α and gene polymorphism of TNF-α*	43 ± 1 years (37 men and 150 women)	A allele in obese patients, also correlated with insulin resistance and HDL cholesterol	Dalziel et al.[25]
5.	Association between serum *TNF-α and gene polymorphism of TNF-α* and IL-6 (mean serum *TNF-α* value in overweight group was 59.70 pg/ml, while mean serum IL-6 value was 122.90 pg/ml)	5—18 years 109 control group (11.00 ± 3.70 years) 79 overweight group (10.10 ± 3.20 years)	Variant genotype (GA or AA) more frequent in normal weight children	Our study

relationship between TNF-α 308 G>A gene polymorphism and glycemia or insulin[9] response. In fact, other studies on Caucasian populations,[33] and even on a Chinese population,[34] have cited correlations between the *TNF-α 308 G* allele and risk of obesity development.

Chang et al. also proved that *TNF-α-308 G>A gene polymorphism* was associated with obesity,[11] whereas Sobti et al. reported that the AA and GA genotypes of *TNF-α 308 G>A* were associated with a higher risk of obesity in men; in women, only the AG genotype was associated with a higher risk of developing this nutritional disorder. The AA and GA homozygotes were also observed to present a higher risk for developing obesity-associated metabolic syndromes.[35] On the other hand, in the present sample, the variant genotype was found to be more frequent among normal weight than overweight children (74.5% versus 25.5%, respectively), emphasizing the fact that the *TNF-α* variant genotype was a protective factor for overweight status (OR = 0.34, 95%CI: 0.13—0.87). Nevertheless, Hedayati did not find any correlation between *TNF-α 308 G>A* gene polymorphism and obesity in an Iranian population.[21] Barchitta et al. noted that women carrying the variant genotype of *TNF-α 308 G>A* gene polymorphism who manifested a weak adherence to the Mediterranean diet had a 3.5-fold increased risk of becoming overweight/obese, similarly to those with a low economic level, who also presented a higher risk of obesity.[36] On the other hand, Brand et al. hypothesized that the G-*308 G>A* polymorphism of the *TNF-α* gene was associated with BMI, therefore representing a genetic marker for increased susceptibility to obesity in the Caucasian population.[9]

To our knowledge, only a limited number of studies have evaluated the relationship of *TNF-α 308 G>A gene polymorphism* with anthropometric and laboratory variables. Patients who carried the *A* allele of *TNF-α 308 G>A* and the IL-6174 CG genotype were reported to have a 2.2-fold higher risk of developing obesity and type 2 diabetes mellitus, therefore underlining the important role of the *TNF-α* gene in predicting the conversion of impaired glucose tolerance in type 2 diabetes mellitus.[37] Other studies have found a race-dependent correlation between the A allele of *TNF-α 308* and obesity, which was present only in the white population between 41 and 60 years old.[38] On the other hand, the same authors observed a significant difference in BMI between GG homozygote and GA + AA genotype associations in the age group between 21 and 40 years.[38]

The above discussion clearly indicates that the data reported in the literature regarding the relationship between *TNF-α 308 G>A gene polymorphism* and obesity are contradictory. In addition, other data supporting a significant negative association between *A*-allele carriers and BMI[39] have been found, a result also indicated by the current finding that the variant genotype (GA or AA) of *TNF-α 308 G>A gene polymorphism* was more frequent among normal weight than overweight children. Therefore, variant genotypes of *TNF-α 308 G>A gene polymorphism* are proven to present a role in the risk of overweight or obesity in humans. However, further studies are needed to clarify whether these variant genotypes have a negative or a positive impact, as well as to identify the precise mechanism of such effect. Most studies hold that these genotypes increase the risk of developing obesity; nevertheless, there are contradictory results in the literature, which, similarly to the current findings, underline the negative impact of these genotypes on weight status.

The present study found no association between the *TNF-α 308 G>A gene polymorphism* and individual biochemical variables. However, the logistic regression analysis carried out to show the influence of the *TNF-α 308 G>A gene polymorphism* influence on overweight risk showed a statistically significant negative effect ($p < 0.001$). The final model comprised five independent variables: *TNF-α* variant genotype, gender, cholesterol, ASAT, and ALAT, which were found to be significant predictors with negative or positive effects on overweight risk in children ($p = 0.024$). The model explained 30% of the variance in the outcome variable and correctly classified 59% of the cases. Contrary to our findings, Sookoian noted that the variant-allele carriers had a 23% risk of developing obesity but also underlined that these individuals were more predisposed to develop higher arterial blood pressure levels and increased insulin levels during the lifetime.[39]

Regarding the association between serum variables and obesity risk, certain studies have reported a positive correlation between *TNF-α*, IL-6, leptin level, and BMI.[6] Contrariwise, the present work found no association between *TNF-α* gene polymorphism and blood glucose levels.

Joffe et al. proved that when the fat intake was 30% of the total energy intake, the obesity rate of *TNF-α* GA + AA—genotype carriers was only 12% compared with GG-genotype carriers. However, when the fat intake was increased, the risk of obesity actually became higher in those with the GA + AA genotype. Therefore, it could be concluded that the *TNF-α 308 G/A gene polymorphism* was able to modify the relationship between dietary fat intake, obesity risk, and serum lipid concentrations in women of color.[40] On the other hand, the variant genotype of this gene polymorphism was found to be more frequent among normal weight than overweight children in the present sample, similarly to the finding reported by Joffe et al. regarding a decreased fat intake. This result is probably due to the fact that the current work focused on children and did not have a large sample.

Nonetheless, certain limitations of this study should be mentioned. First, the sample included a relatively small number of obese/overweight children (n = 79) and controls (n = 109), which decreased the statistical power of the research, as well as explains the lack of certain correlations with other results reported in the literature. Considering the fact that the investigated genotypes in the overweight children were not in Hardy—Weinberg equilibrium, the obtained results should be interpreted with caution. One explanation for this deviation from Hardy—Weinberg equilibrium may be the very small number of cases (only 4) with variant homozygous genotypes in our study. Nevertheless, to our knowledge, this research is the first to assess overweight risk in children. There are no data in the literature regarding the association between *TNF-α 308 G>A gene polymorphism* and overweight risk in children; hence, this work is regarded as a pilot study that should be expanded to a larger population of children.

Another limitation is that the serum levels of leptin and adipokines, and the intake of proteins, carbohydrates, and lipids in this group were not evaluated. In addition, the

obesity classification according to body composition was not applied because the BMI does not always measure body fat directly and poorly distinguishes between fat mass and lean or bone mass. Further, a relatively uniform Caucasian population from central Romania was chosen, without information on whether the environment, geographic conditions, or eating habits might influence the results. A study of the same gene polymorphism in the subjects' parents is also highly recommended in the future.

Nevertheless, this study also has several strong points, such as the relatively uniform Caucasian population; the assessment of overweight risk in children, as opposed to most studies, which assessed obesity risk; and the specific investigation of the impact of one of the most controversial gene polymorphisms on overweight risk in children. Further studies in bigger cohorts are recommended to establish more clearly the role of *TNF-α 308 G>A gene polymorphism* in predicting children's overweight risk.

5. Conclusion

Children's overweight can be considered as a modifiable nutritional disorder, providing the possibility to prevent the potential complications of obesity. Therefore, it is essential to identify the overweight risk in children toward preventing its subsequent, or otherwise inevitable, development into obesity. According to our findings, overweight children are at a higher risk of presenting increased cholesterol, LDL cholesterol, GPT, and glycemic levels compared with normal weight children. In the studied sample, the variant genotype of *TNF-α 308 G>A gene polymorphism* was more frequently found among normal weight children. In addition, adjusting for the presence of some covariates, such as age, gender, cholesterol, ASAT, and ALAT, *TNF-α 308 G>A gene polymorphism* remained as an independent predictor with a negative effect on children's overweight risk. These findings support the hypothesis that *TNF-α 308G>A gene polymorphism* might be involved in child's overweight status.

Conflict of interest

None of the authors declare any conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.03.003.

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