

## Implications of visfatin genetic variants in the metabolic profile of the Romanian pediatric population

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### Abstract

**Background:** Conflictual results regarding the relationship between plasmatic level of visfatin and obesity could be explained by the influence of the gene variants involved in the synthesis or action of these hormones. **Objectives:** The present study examined the potential implication of single nucleotide polymorphisms (SNPs) of nicotinamide phosphoribosyltransferase (NAMPT) gene that encodes visfatin, in obesity, in a Romanian pediatric population. **Method:** A case-control study was conducted on a group of 213 children, divided into two: the case group - 130 overweight and obese children with BMI >1 SD, and the control group - 83 children with normal BMI. The variables analyzed were age, sex, anthropometric parameters, body composition based on bioimpedance analysis, lipid profile, visfatin and insulin plasmatic levels, rs4730153 and rs2302559 visfatin SNPs. **Results:** Significant associations were not found between rs4730153 and rs2302559 visfatin SNPs and obesity. Regarding lipid metabolism, there are statistically significant differences between triglyceride levels according to NAMPT rs2302559 genotypes

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( $p=0.045$ ), with heterozygous genotype having the highest level of triglycerides, and also between cholesterol levels according to NAMPT rs4730153 genotypes ( $p=0.030$ ), with carriers of heterozygote genotype having the highest level of cholesterol. There is a statistically significant difference between the studied parameters in the investigated groups, regarding cholesterol, in carrier of wild-type genotype of NAMPT rs2302559 ( $p=0.040$ ) and carrier of wild-type genotype of NAMPT rs4730153 ( $p=0.036$ ). We observed no association of NAMPT rs4730153 and rs2302559 with visfatin levels in the studied groups. Visfatin level was lower in the case group and was correlated with weight ( $p=0.042$ ), abdominal circumference ( $p=0.010$ ), waist to height ratio ( $p=0.040$ ), but not with the elements of the metabolic profile. **Conclusion:** NAMPT rs2302559 and rs4730153 polymorphisms do not seem to have a major impact in the development of obesity in children, however there may be an association with lipid profile, but further studies are needed..

**Keywords:** visfatin, insulin, visfatin polymorphisms, pediatric obesity

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## Introduction

Obesity in children, adolescents, and adults is considered to be the most important public health problem in recent years (1, 2). The World Health Organization (WHO) estimates that in 2025 approximately 70 million children will be overweight or obese unless adequate prevention and control measures are applied. Romania occupies the third place in Europe in terms of obesity incidence according to IOTF (International Obesity Task Force) (3-5). The PREDATORR study showed an increase in the prevalence of obesity in the adult population in Romania. The study performed on 2681 patients, a cohort statistically representative for the entire adult population of Romania, revealed that the prevalence of obesity was 31.90% and the prevalence of abdominal obesity was 73.90% (6). If in 2009 15% of Romanian children were obese, in 2015 the percentage increased to 25%. A pooled analysis of data on body mass index BMI disturbances in Romanian children from 2006 to 2015 revealed that the prevalence of children with obesity was 28.3%, with the highest prevalence in children 6-12 years of age (34%) and the lowest in the 15-19 year-old group (17%) (7).

Based on the latest research, the American Association of Clinical Endocrinologist (AACE)

and the American College of Endocrinology (ACE) proposed a new terminology for obesity: adiposity-based chronic disease (ABCD). Many researchers have extensively studied the adipose tissue, however, the attention has been increasingly focused on the term “adiposity”, which includes the function, distribution and the total quantity of adipose tissue (8).

Although it was known that adipose tissue secreted peptides, the number of adipokines has increased rapidly and includes adiponectin, resistin, visfatin, retinol binding protein 4 (RBP4), and chemerin. The secretion and preferential expression of pro-inflammatory molecules (visfatin, RBP4) was demonstrated in the adipose tissue of adult obese persons. All these adipokines have an important role in lipid homeostasis, blood pressure control, insulin sensitivity, coagulation, and have an essential contribution to obesity caused complications (9).

Insulin resistance (IR) appears to be an important disorder in the pediatric population. The pathway by which IR occurs is unclear, but studies have categorically argued that patients with IR are susceptible to metabolic syndrome development, type 2 diabetes and cardiovascular disease (10). The homeostasis model assessment for insulin resistance (HOMA-IR) index

is generally used for defining IR in the adult population and has been applied in the pediatric population as well (11).

Visfatin was discovered as a growth factor for B lymphocyte precursors. It is encoded by the nicotinamide phosphoribosyltransferase (*NAMPT*) gene located on the long arm of chromosome 7. It is an adipokine secreted by the visceral adipose tissue, but also by macrophages within the adipose tissue, hepatocytes, as well as skeletal muscles (12-14), and it has been suggested that it might have both endocrine and paracrine effects. The hypothesis regarding the role of macrophages in the production of visfatin is supported by differences in visfatin production in the visceral and subcutaneous adipose tissue. It appears that RNA expression is identical in the visceral and subcutaneous adipose tissue in normal weight patients. Differences occur in obese patients, where visceral adipose tissue becomes a more potent visfatin producer, while it decreases at subcutaneous level (12). It was reported that visfatin acts on the insulin receptor by binding to another site than that of the usual substrate, but this hypothesis is still under debate (15).

In the pediatric population, the metabolic significance of visfatin remains unclear. The conflicting results regarding the relationship between insulin plasmatic levels as well as visfatin and obesity could be explained by the influence of the different gene variants involved in the synthesis or action of these hormones.

The latest studies have shown a genomic association of 227 gene variants involved in many biological pathways (appetite and digestion, adipocyte differentiation, insulin pathways, intestinal microbiota), and obesity (16). The link between some visfatin gene variants and obesity has been confirmed in several populations, but not in Romanian children (17). For the present study we selected rs4730153 and rs2302559 visfatin gene polymorphisms because there are studies in the adult population that have shown their involve-

ment in the metabolic profile. However, studies in the pediatric population are extremely limited and the visfatin gene polymorphisms we have chosen have not been studied in the Romanian pediatric population. Thus, given the high prevalence of pediatric obesity in our country, *the aim* of the current study was to evaluate the associations between two single nucleotide polymorphisms (SNPs) for visfatin rs4730153 and rs2302559, and other metabolic abnormalities in overweight, obese, and normal weight children.

## Materials and Methods

### *Study design*

An observational study was conducted on 213 children, from three schools from Târgu Mures, Pediatrics Clinic no.1, and Endocrinology Clinic from Targu Mures, aged between 5 and 18 years, divided into two groups: the study group - 130 overweight and obese children with BMI >1 SD and the control group - 83 children with normal BMI, who were evaluated between 2016 and 2018. Inclusion criteria were: age between 5 and 18 years; study group: overweight (BMI >+1 SD, according to the WHO) (18) or children with obesity (BMI >+2 SD); control group (BMI SD between -1 and +1 SD). Exclusion criteria were: history of severe chronic disease (cardiac disease, liver failure, renal insufficiency or malabsorption syndromes: celiac disease, short bowel syndrome, inflammatory bowel disease, as well as any known monogenic or chromosomal genetic diseases), children with short stature (height  $\leq$ -2.5 SD (based on the Prader, 1989 growth charts) (19), untreated endocrine disease, failure to obtain informed consent from the parents.

All parents signed a written consent for their children. The study was approved by the Ethics Committee of the University (No. 7/2016) and it was compliant with the principles of the Helsinki Declaration.

### Method

Upon written consent, all children were auxologically evaluated: height, weight, waist circumference, tricipital skinfold, using standardized tools and measurement protocols (20). The second step was the bioimpedance analysis after which blood was drawn for biochemical and genetic analysis.

The following variables were included: age, sex, anthropometric parameters: standard deviation score (SD) of body mass index (BMI), abdominal circumference (AC), tricipital skinfold thickness (TST), waist to height ratio (WHtR), body composition based on bioimpedance analysis (fat mass, free-fat mass), blood glucose, lipid profile (total cholesterol, HDL cholesterol, triglycerides), uric acid, visfatin and insulin plasma levels, HOMA index, 2 SNPs for visfatin namely, rs4730153 and rs2302559.

For waist circumference, the mean of two values was used. The position of the tape was approximately the midpoint of the line between the top of the iliac crest and the lower margin of the last palpable rib, (21). The available reference values were used (22) defining abdominal obesity as values above as previously described +2 SD. The waist circumference/height ratio was used as a second definition for abdominal obesity with a cut-off value of 0.5 (22). Tricipital skinfold thickness was measured from the left arm, using a digital caliper, with a precision of 0.1 mm, and was interpreted as normal -1 SD - +1 SD, overweight +1 SD - +2 SD and obesity  $\geq$  +2 SD (22).

Body composition based on bioimpedance analysis (using the Tanita BC-420 analyzer): the principle of this method is to measure the electrical resistance of the tissues of the body, determining the values corresponding to the quantity and percentage of each tissue type. Interpretation: for children under 16 years of age, obesity is defined as fat mass that exceeds the reference value for age and gender by more than 20%; for

children over 16 years the cut-off value for fat mass is 25% of the body weight in boys and 32% in girls (23).

Metabolic parameters analyzed: glucose levels; lipid profile: triglycerides, cholesterol, HDL cholesterol, uric acid. The biological tests for metabolic determinations were performed in the morning, after a fasting period of at least 8 hours. The reference range for all these metabolic parameters was established according to the cut-off values of the hospital.

Blood collection for biomarker assessment was performed in EDTA tubes. The samples were centrifuged for 15 mins at 1500g, aliquoted in safe-lock tubes and stored at -80 °C until analysis. Visfatin plasma levels (Visfatin, Sigma-Aldrich, USA) and insulin (Human Insulin, DRG Instruments Germany) were measured on an automated DSX ELISA instrument (Dyner Technologies, USA) using the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions and as previously described (24). The analytical sensitivity for Insulin was 1.76  $\mu$ UI/ml and coefficients of variation (CVs) for intra-assay and inter-assay precision were  $<$  2.6% and  $<$  3.0% respectively HOMA index (calculated with formula (glycemia x insulin)/405) (25), was interpreted as:  $<$ 2 normal,  $>$ 2 possible insulin resistance,  $>$ 2.5 increased probability of insulin resistance (26). For visfatin, the minimum detectable concentration was 0.778 ng/ml, while CVs were  $<$ 10% for intra-assay precision and  $<$ 15% for inter-assay precision.

Genotyping analysis: the Quick-DNA miniprep kit (ZymoResearch, USA) was used for processing fresh whole blood collected in EDTA from children in order to obtain the genomic DNA which was used for genotyping analysis. Two SNPs for visfatin within the *NAMPT* gene, namely rs4730153 (-3186C>T) and rs2302559 (-948G>T) were genotyped using specific TaqMan assays and an Applied Biosystems 7500

Fast Dx Real-Time PCR instrument from ThermoFisher Scientific (USA).

Statistical analysis: for data collection, the Microsoft Office Excel software was used. The statistical analysis was performed using SPSS v. 25.0 (SPSS Inc, Chicago, IL, USA). For data display of continuous variables, the mean  $\pm$  standard deviation was used for normally distributed data, and median and interquartile range for non-normally distributed data. Categorical variables were expressed as frequencies and percentages. For mean comparison, the Mann-Whitney test was used; associations were tested using Fisher's exact test. Deviations from the Hardy-Weinberg equilibrium for genotype frequencies were tested using the chi-square test. Spearman coefficient was used for correlation analysis. For all analysis, statistical significance was obtained if  $p < 0.05$ .

## Results

### *Demographic characteristics*

The general characteristics and metabolic profile

of the studied groups are described in Table 1, with no significant differences between median age and sex ratio in the two groups. Median age of the case group was 11.5 years (9.1-13.4) and 12.0 years for the control group (9.1-14), with a sex ratio Male:Female = 1.1 for the case group and 0.8 for the control group ( $p = 0.262$ ).

There were significant differences between the two groups regarding the anthropometric parameters, all body composition parameters and metabolic parameters (cholesterol, triglyceride, HDL cholesterol, uric acid, insulin, HOMA index and visfatin), however, regarding glucose level there was no significant difference between the two groups ( $p = 0.188$ ) (Table 1).

### *Hormonal characteristics*

The HOMA index was significantly higher in the case group compared with the control group (7.58 vs 4.66,  $p < 0.001$ ). The HOMA index was significantly correlated with weight ( $r_s = 0.355$ ,  $p = 0.001$ ), BMI ( $r_s = 0.390$ ,  $p = 0.001$ ), abdominal circumference ( $r_s = 0.386$ ,  $p = 0.001$ ), WHtR ( $r_s = 0.333$ ,  $p = 0.001$ ), skinfold ( $r_s = 0.230$ ,

**Table 1. Anthropometric and metabolic comparison between study groups**

	Case (BMI>1 SD)	Control (BMI<1 SD)	p value
BMI SD	+2.48	-0.10	<0.001
Waist SD	+2.54	+0.75	<0.001
WHtR	0.58	0.46	<0.001
TST SD	+2.97	-0.54	<0.001
Fat-Mass (kg)	22.44	7.56	<0.001
Free Fat-Mass (kg)	39.02	32.94	0.001
Muscle Mass (kg)	37.64	32.01	0.003
Glucose (mg/dl)	82.67	81.08	0.188
Cholesterol (mg/dl)	166.99	156.98	0.049
Triglycerides (mg/dl)	89.87	71.24	<0.001
HDL-Cholesterol (mg/dl)	51.09	55.46	0.001
Uric Acid (mg/dl)	4.91	4.23	<0.001
Insulin ( $\mu$ UI/ml)	37.01	23.47	<0.001
HOMA index	7.58	4.66	<0.001
Visfatin (ng/ml)	13507.13	16504	0.014

BMI: body mass index, HDL: high density lipoprotein, HOMA index: homeostasis model assessment for insulin resistance index, statistical significance was obtained if  $p < 0.05$ , SD: standard deviation, TST: tricipital skinfold thickness, WHtR: waist per height ratio, Values are expressed as mean.

$p=0.001$ ), fat-mass ( $r_s=0.473$ ,  $p=0.001$ ), free fat-mass ( $r_s=0.378$ ,  $p=0.001$ ), muscle mass ( $r_s=.344$ ,  $p=0.001$ ), glucose ( $r_s=0.281$ ,  $p=0.001$ ), triglycerides ( $r_s=0.335$ ,  $p=0.001$ ), HDL-cholesterol ( $r_s=-0.166$ ,  $p=0.033$ ). The HOMA index and insulin showed correlations with the same studied parameters, however, in addition to the insulin level, the HOMA index was significantly correlated with uric acid ( $r_s=0.181$ ,  $p=0.020$ ). There was no significant correlation between the HOMA index and total cholesterol and visfatin level.

Visfatin level was lower in the case group (13507 vs 16504 ng/ml,  $p<0.014$ ) and was correlated with weight ( $r_s=.159$ ,  $p=0.042$ ), abdominal circumference ( $r_s=-0.200$ ,  $p=0.010$ ), WHtR ( $r_s=-0.158$ ,  $p=0.040$ ), but not with metabolic profile elements (total cholesterol, triglycerides, fasting glucose, insulin level and HOMA index).

### Genetic characteristics

The allele frequency corresponded to the general data for each polymorphism, and the genotype frequency was in Hardy-Weinberg equilibrium

(for *NAMPT* rs2302559  $\chi^2=0.3$ ,  $p=0.060$ ; *NAMPT* rs4730153  $\chi^2=0.17$ ,  $p=0.069$ ), however, significant associations were not found between the investigated visfatin SNPs and obesity (Table 2).

There was no significant difference regarding the anthropometric parameters (AC  $p=0.238$ , WHtR  $p=0.231$ ), body composition elements (fat mass  $p=0.402$ ), metabolic parameters (HOMA index  $p=0.943$ ), and visfatin ( $p=0.147$ ) for the *NAMPT* rs4730153 genotypes (wild-type homozygous genotype AA, heterozygous genotype AG and variant homozygous genotype GG). Considering lipid metabolism, there were statistically significant differences between plasma cholesterol levels according to *NAMPT* rs4730153 ( $p=0.030$ ) with AG genotype showing the highest level of cholesterol.

*NAMPT* rs4730153 wild-type homozygous genotype (AA) carriers presented no significant difference between the studied parameters in the investigated groups: anthropometric parameters (BMI  $p=0.803$ , AC  $p=0.617$ ), body composition elements (fat mass  $p=0.691$ ), metabolic pa-

**Table 2. Genotype distribution of visfatin rs2302559 and rs4730153 SNPs**

<i>NAMPT</i> rs2302559	Case (BMI>1 SD) N=130	Control N=83	OR (95%CI), p value	Allele frequencies %
C	112	70	1.26 (0.77-2.06), $p=0.386$	66.18
T	77	38	-	33.81
CC	49	43	1.11 (0.46-2.72), $p=0.825$	
CT	63	27	0.54 (0.22-1.35), $p=0.231$	
TT	14	11	-	
CT+CC	112	70	0.79 (0.34-1.85) $p=0.663$	
<i>NAMPT</i> rs4730153				
A	89	45	-	39.85
G	107	69	1.27 (0.79-2.03) $p=0.3423$	60.14
AA	19	12	-	
AG	70	33	0.74 (0.32-1.71), $p=0.519$	
GG	37	36	1.54 (0.65-3.62), $p=0.391$	
GG+AG	107	69	1.02 (0.46-2.23), $p=0.999$	

*Visfatin* rs2302559: C – variant allele; CC – variant homozygous genotype, TT – wild-type homozygous genotype; CT – heterozygous genotype; T – wild-type allele; *Visfatin* rs4730153: A – wild-type allele; AA – wild-type homozygous genotype, GG – variant homozygous genotype; AG – heterozygous genotype; BMI: body mass index; G – variant allele; SD: standard deviation;

rameters (triglycerides  $p=0.922$ , HOMA index  $p=0.931$ ), and visfatin ( $p=0.170$ ). Considering cholesterol levels, there was a statistically significant difference between the two groups in *NAMPT* rs4730153 AA carriers ( $p=0.036$ ).

*NAMPT* rs4730153 heterozygous genotype (AG) carriers presented no significant difference between the studied parameters in the investigated groups: anthropometric parameters (BMI  $p=0.526$ , AC  $p=0.072$ ), body composition elements (fat mass  $p=0.310$ ), metabolic parameters (triglycerides  $p=0.056$ , HOMA index  $p=0.660$ ), and visfatin ( $p=0.150$ ). However, when considering cholesterol levels, there was a statistically significant difference between the two groups in *NAMPT* rs4730153 AG carriers ( $p=0.031$ ).

*NAMPT* rs4730153 variant homozygous genotype (GG) carriers presented no significant difference between the studied parameters in the investigated groups: anthropometric parameters (BMI  $p=0.247$ , AC  $p=0.188$ ), body composition elements (fat mass  $p=0.188$ ), metabolic parameters (cholesterol  $p=0.452$ , triglycerides  $p=0.051$ , HOMA index  $p=0.868$ ), and visfatin level ( $p=0.171$ ).

There was no significant difference regarding the anthropometric parameters (in case of abdominal circumference  $p=0.218$ , WHtR  $p=0.269$ ), body composition elements (fat mass  $p=0.186$ ), metabolic parameters (HOMA index  $p=0.703$ ), and visfatin ( $p=0.460$ ) for the *NAMPT* rs2302559 genotypes (wild-type homozygous genotype TT, heterozygous genotype CT and variant homozygous genotype CC). Considering lipid metabolism, there were statistically significant differences between plasma triglyceride levels according to *NAMPT* rs2302559 ( $p=0.045$ ), with CT genotype having the highest triglyceride level.

*NAMPT* rs2302559 wild-type homozygous genotype (TT) carriers presented no significant difference between the studied parameters in the investigated groups: anthropometric parameters

(BMI  $p=0.760$ , AC  $p=0.400$ ), body composition elements (fat mass  $p=0.995$ ), metabolic parameters (HOMA index  $p=0.870$ ), and visfatin ( $p=0.160$ ). For cholesterol levels, there was a statistically significant difference between the two groups of *NAMPT* rs2302559 homozygous TT genotype carriers ( $p=0.040$ ).

*NAMPT* rs2302559 heterozygous genotype (CT) carriers presented no significant difference between the studied parameters in the investigated groups: anthropometric parameters (BMI  $p=0.237$ , AC  $p=0.067$ ), body composition elements (fat mass  $p=0.082$ ), metabolic parameters (HOMA index  $p=0.404$ ), and visfatin ( $p=0.883$ ). Considering the triglycerides levels, there was a statistically significant difference between the two groups of *NAMPT* rs2302559 heterozygous CT genotype carriers ( $p=0.015$ ).

*NAMPT* rs2302559 variant homozygous genotype (CC) carriers presented no significant difference between the studied parameters in the investigated groups: anthropometric parameters (BMI  $p=0.273$ , AC  $p=0.283$ ), body composition elements (fat mass  $p=0.196$ ), metabolic parameters (cholesterol  $p=0.387$ , HOMA index  $p=0.791$ ), and visfatin ( $p=0.165$ ).

## Discussions

Childhood is considered a critical period for the onset of obesity which in turn leads to significant health changes with negative impact on adult's health (27).

The interaction between HOMA-IR and BMI in preadolescence predicts the likelihood of having metabolic syndrome in late adolescence. There are studies that showed a significant positive correlation between insulin levels, HOMA index and weight, BMI, AC and WHtR (28, 29), in concordance with our results. Research demonstrated that increased insulin levels were an independent risk factor for the appearance of cardiovascular conditions, by increasing the risk

of dyslipidemia (30), among those with hyperinsulinemia, dyslipidemia cases being threefold higher (27). In our study, the HOMA index was significantly correlated with triglycerides, but there was no significant correlation with total cholesterol. Our results were similar to the study performed by Romualdo et al. which revealed that triglyceride levels were significantly higher in participants with insulin resistance, but there was no significant correlation between HOMA index and total cholesterol (31).

Serrano et al. demonstrated that overweight adolescents were 4.5-fold more likely to have changes in the HOMA index, while adolescents with higher fat mass percentage had higher HOMA index values (32). These data support our results, as in our study insulin plasmatic levels and HOMA index were correlated with all body composition components – fat mass, non-fat, and muscle mass. There is still no agreement on the normal values of HOMA-IR for pediatric evaluation, as these values tend to fluctuate greatly, especially at puberty. To more accurately investigate the relationship between HOMA-IR cut-off and associated risk, more prospective studies need to be considered.

Studies which evaluated visfatin plasma levels are highly controversial in both adults and pediatric populations. In our study, visfatin levels were significantly lower in the obese and overweight group compared with the control group, contrary to other studies that showed elevated visfatin levels in obese or overweight patients (33-35), nevertheless these studies included children with obesity and at least three criteria for metabolic syndrome (34) or they were performed on small sample populations (35). However, our results were similar to those of a study conducted on 241 newly diagnosed type 2 diabetes patients and normal glucose tolerant adult subjects which showed that visfatin levels were significantly lower in patients with obesity compared with overweight and normal-weighted subjects (36).

In children, studies have shown a positive correlation between visfatin plasma concentrations and BMI and abdominal circumference (34-36). Similarly, in our study visfatin plasmatic levels were also correlated with BMI, AC, weight, and WHtR. It has been discovered that visfatin levels increase with the number of components of the metabolic syndrome (34). However, our study showed that visfatin plasmatic levels were not correlated with metabolic parameters (cholesterol, triglycerides, glucose or HOMA index), even though in an obese adult population serum visfatin levels were negatively correlated with HDL and positively correlated with serum triglycerides (38).

There are studies suggesting that insulin might be a potential mediator in the visfatin pathway, and there is a stronger link between insulin and visfatin than glucose (39), although other studies revealed controversial results on the expression of visfatin and its relationship with insulin resistance (35, 40). The discordance between results could be attributed to the differences in sensitivity and specificity of the available visfatin immunoassay kits. However, in our study, there was no significant correlation between these parameters. Conflicting results considering the relationship between visfatin plasmatic levels and obesity could also be explained by the influence of gene variants involved in the synthesis or action of these hormones. However, literature data about these polymorphisms are controversial and extremely limited.

Considering *NAMPT* rs2302559, our results showed no significant differences between its genotypes and anthropometric parameters (BMI, AC, WHtR, fat mass), results that are supported by other studies (40). Considering visfatin level, a study which analyzed 243 obese children showed that rs2302559 variant allele carriers had lower fasting serum visfatin and lower fasting plasma glucose as compared with wild-type allele carriers (39), however, in our study, there



was no significant difference in visfatin levels between *NAMPT* rs2302559 genotypes.

Regarding the lipid metabolism, in our study, there were statistically significant differences between plasma triglyceride levels according to *NAMPT* rs2302559 genotypes, with the heterozygous genotype having the highest level of triglycerides. We did not find similar results in the literature.

Considering the *NAMPT* rs4730153 genotypes, regarding the lipid profile, we found statistically significant differences between plasma cholesterol levels, with carriers of the heterozygote genotype having the highest cholesterol levels, results supported by another study indicating that the same polymorphism was likely to contribute to lipid metabolic malfunction (41). However, these results disagree with other previous studies in which no association between rs4730153 SNP and cholesterol, HDL cholesterol and triglycerides was found (42, 43).

In our study, no associations were found between *NAMPT* rs4730153, anthropometric and body composition parameters, HOMA index and visfatin levels, results supported by other studies (42). However, there was a study on the adult population that showed a significant association of *NAMPT* rs4730153 variant allele with glucose, insulin and HOMA index (15). In our study, *NAMPT* rs4730153 variant genotype was not associated with anthropometric parameters, HOMA index or visfatin levels. The reason for the discrepancies among these studies is unclear, but differences in race, the number of the children investigated in different studies, and determination techniques (visfatin immunoassay kits) might contribute to the discordant results.

Finally, some study limitations must be highlighted: obesity is a multifactorial problem (44); genetic, metabolic, social and hormonal factors, lack of physical activity, unhealthy regional eating habits being the most commonly involved ones. We did not evaluate puberty in our chil-

dren, a physiological stage known to modify insulin resistance. Also, in the control group, we included children referred to the hospital for minor conditions that might have other characteristics which influence visfatin levels. To the best of our knowledge, our analysis is the first study which analyzes the Eastern European pediatric population. Although this study demonstrated an association between visfatin rs4730153 and rs2302559 SNPs and metabolic factors, the specific pathway by which the studied SNPs influence the cardiometabolic parameters remain to be established by future studies.

**In conclusion,** *NAMPT* rs2302559 and rs4730153 polymorphisms do not seem to have a major impact in the development of obesity in children. However, there may be an association with lipid profile, but further studies in order to confirm the association at populational levels are needed. Additional genetic studies in larger study groups are required to perform the profile of these polymorphisms and to confirm the association at populational levels.

## Abbreviation

SNP: single nucleotide polymorphisms

WHO: World Health Organization

IOTF: International Obesity Task Force

AACE: American Association of Clinical Endocrinologist

ACE: American College of Endocrinology

ABCD: Adiposity-based chronic disease

RBP4: retinol binding protein 4

IR: insulin resistance

HOMA – IR: homeostasis model assessment for insulin resistance index

*NAMPT*: nicotinamide phosphoribosyltransferase

RNA: Ribonucleic acid

BMI SD: body mass index standard deviation score

AC: abdominal circumference

SD: standard deviation score  
 TST: Tricipital skinfold thickness  
 WHtR: waist to height  
 HDL: high-density lipoprotein  
 EDTA: Ethylenediaminetetraacetic acid  
 CVs: coefficients of variations  
 OD: odds ratio  
 CI: confidence interval

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## Author Contributions

SLV (Conceptualization, Investigation, Writing-original draft presentation);  
 IMP (Conceptualization, Resources, Writing-review and editing);  
 AB (Investigation)  
 RMP (Formal analysis, Writing-review and editing);  
 CB (Genotyping, Conceptualization, Resources, critical review of the manuscript and editing);  
 VM (DNA isolation, Genotyping, Resources);  
 AH (Visfatin quantification, Resources);  
 CD (Sample and data collection, Writing-review and editing);  
 COM (Conceptualization, Resources, Writing-review and editing).

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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