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Association between rs4994 variant in *β3-Adrenergic receptor* and obesity in Vietnamese preschool-age children, independent of eating behaviors

Nguyen Thi Hong Hanh[1], Do Thi Nhu Trang[1], Nguyen Thi Trung Thu[1] and Le Thi Tuyet[1*]

Abstract

Background The Arg64 allele of the rs4994 (Trp64Arg) variant in the *β3-adrenergic receptor* (*ADRB3*) gene is involved in the control of energy balance by altering lipolysis and thermogenesis in adipocytes, ultimately contributing to the development of obesity. The objective of our study was to investigate the association between the rs4994 variant of the *ADRB3* gene and obesity in Hanoi preschool-age children, adjusting for their eating behaviors.

Methods A cross-sectional study was performed involving 708 children with normal weight and 304 children with obesity aged 3–5 years from 36 kindergartens in Hanoi, Vietnam. Cheek mucosa cell samples were used for DNA extraction, and genotyping at the *ADRB3*-rs4994 locus was performed using the polymerase chain reaction–restriction fragment length polymorphism method (PCR–RFLP). Eating behaviors were assessed using the Children's Eating Behaviour Questionnaire (CEBQ). Binary logistic regression analysis was employed to examine the association between the rs4994 variant and obesity, adjusting for confounding factors such as age, sex, residence, birth weight, and eating behaviors.

Results The frequency of the C allele in the group with obesity was 16.4%, which was higher than in the control group (11.7%, $P=0.003$). Children with the CC genotype exhibited significantly greater weight and weight-for-age Z-score compared to those with the TT and TC genotypes ($P=0.004$ and 0.03, respectively). Following univariate and multivariate analyses adjusted for age, sex, residence, birth weight, and eating behaviors, a significant association between the rs4994 variant and obesity was observed ($P<0.05$).

Conclusions This study indicated that the *ADRB3*-rs4994 variant can be considered as an independent risk factor for obesity in Vietnamese preschool children.

Keywords *ADRB3*, Obesity, Genetic variance, rs4994, Vietnamese children

*Correspondence:
Le Thi Tuyet
tuyetlt@hnue.edu.vn
[1]Hanoi National University of Education, 136 Xuan Thuy Street, Hanoi, Vietnam

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Background

The prevalence of childhood obesity has remarkably increased worldwide in recent decades. Within 40 years (1975–2016), the number of school-age children and adolescents with obesity rose more than 10-fold, from 11 million to 124 million [1]. Obesity had been considered a huge problem in high-income countries before, but now it is also a serious problem in low-income countries, especially in urban areas [2]. The childhood obesity rate in developing countries has increased by more than 30% compared to developed countries [3].

According to data from World Health Organization (WHO), childhood obesity has become globally one of the biggest health threats of the 21st century due to its serious consequences [3]. Childhood obesity adversely affects not only the physical health but also the mental development of children [4]. Obesity is a major factor that contributes to metabolic syndrome, hypertension, type II diabetes mellitus, dyslipidemia, and cardiovascular diseases, and makes children feel an inferiority complex and more likely to engage in high-risk behaviors such as smoking or drinking alcohol [5].

The incidence of overweight and obesity is on the rise among children of all age groups in Vietnam [6, 7]. A cross-sectional study conducted in 2018 showed that overweight and obesity were emerging challenges among preschool Kinh ethnic children, with 10.3% of boys and 5.9% of girls experiencing overweight or obesity [6]. In 2020, the prevalence of overweight-for-height among infants and children under the age of 5 years was 7.4%. Among children and adolescents aged 5–19 years, the prevalence of overweight was 19.0% and the prevalence of obesity was 8.1% [7].

Obesity is a multifactorial syndrome influenced by both genetic and environmental factors. To create a long-term and effective way to prevent this morbidity, it is essential to have a comprehensive understanding of factors that contribute to childhood obesity [8].

Obesity is the result of an imbalance between food intake and energy consumption, which is closely related to lifestyle and dietary intake preferences [9]. One of the factors that are closely related to childhood obesity is eating behavior. Steinsbekk et al. (2017) also showed that fat mass and muscle mass were strongly associated with specific eating behaviors [10]. A study was conducted on 2,049 preschool children aged 3–6 years in Taizhou, China, in 2022, utilizing the Children's Eating Behavior Questionnaire. The findings indicate that within the "Food Avoidant" subscales, satiety responsiveness ($P<0.001$) and slowness in eating ($P=0.001$) scores were inversely correlated with body mass index Z-scores among preschool children of both genders. In the "Food Approach" subscales, the enjoyment of food score exhibited a positive association with body mass index Z-score in both boys ($P=0.007$) and girls ($P=0.035$). Additionally, the association between food responsiveness scores and body mass index Z-score was observed exclusively in girls ($P=0.001$) [11]. Besides that, many other factors have been demonstrated that increase the risk of childhood obesity such as socioeconomic status [12], physiological, metabolic, and genetic factors [13, 14]. Our study in 2017 suggested a significant association between delivery method, birth weight, night sleep duration, and *BDNF* Val66Met variant, with obesity in Vietnamese primary school children [15].

In recent years, many studies have focused on the β_2 *adrenoreceptor* and β_3 *adrenoreceptor* genes of the adrenergic receptor system, which play an important role in stimulating thermogenesis and lipid mobilization in adipose tissues. The β3-adrenergic receptor is expressed in visceral adipose tissue and plays a pivotal role in the regulation of human brown/beige adipocyte lipolysis and thermogenesis [16]. In 1997, the *ADRB3*-rs4994 variant, characterized by a T to C substitution resulting in the replacement of tryptophan by arginine at position 64 (Trp64Arg), was independently reported by three different groups [17]. As a crucial component for regulating energy balance in mammals, the essential role of *ADRB3* gene is due to its effects on lipid degradation, fatty acid transport, thermogenesis [18, 19]… Binding to β_3-adrenergic agonists leads to the activation of *ADRB3*, which then activates adenylyl cyclase. Activated adenylyl cyclase initiates a signal pathway through intracellular signaling cascades including cyclic AMP, and kinase proteins. ultimately resulting in heat production in brown and white adipocytes [20, 21]. According to the research by Krief et al. (1993), *ADRB3 gene* also participates in the regulation of lipid metabolism including the absorption of fat during digestion, storage, and mobilization of lipids from adipocytes [18]. Thus, dysfunction of *ADRB3* can impair the processes of lipolysis and energy expenditure, which may contribute to the development of obesity and metabolic disorders through the excessive accumulation of fat in adipose tissue [22].

According to some previous studies, the rs4994 variant of the *ADRB3* gene is one of the candidates for obesity. A meta-analysis from 16 different studies indicated that this variant might considerably contribute to both obesity in children and adults, particularly in populations belonging to the East Asia regions [23]. Up to now, there has been a paucity of published reports about the association between the *ADRB3*-rs4994 variant and childhood obesity in Vietnamese populations.

Therefore, to prove whether the above results are correct for the Vietnamese population or not, the study was conducted to determine the association between the rs4494 variant of the *ADRB3* gene and obesity in 3-5-year-old Vietnamese children.

We carried out this study to inquire into the association between the rs4994 variant belonging to the *ADRB3* gene and childhood obesity, adjusted for children's eating behavior.

Results

Table 1 showed the values for anthropometric measurements, genotypes and allele frequencies of *ADRB3*-rs4994 in the non-obese and obese groups. There was no significant difference in gender, age, or weight between the two groups ($P>0.05$). Meanwhile, height, BMI, height-for-age Z-score, weight-for-age Z-score, weight-for-height Z-score, and BMI-for-age Z-score of cases were significantly higher than those of controls ($P<0.05$).

Results from Table 1 indicated that the genotype and allele frequencies were statistically different between the two groups ($P=0.016$ and $P=0.003$, respectively). For the entire study sample, the TT genotype had the highest frequency (78.4% in controls, 71.5% in cases). The frequency of CC genotype in the group with obesity was twice as much as the group with normal weight (4.2% and 2.0%, $P=0.016$). Although both groups were in Hardy-Weinberg equilibrium ($P=0.134$ for the control group and $P=0.059$ for the case group, *P* value acquired from Fisher's exact test), there was a significant difference in allele frequencies between two groups ($P=0.003$). In group with obesity, the frequency of allele C was 16.4%, which was higher compared with 11.7% in group with normal weight.

Table 2 showed the characteristics of obesity-related traits in total, case, and control groups. In the overall sample, distinctions in weight and weight-for-age Z-score were observed based on the genotype at the *ADRB3*-rs4994 variant. Specifically, children with the CC genotype exhibited significantly greater weight and weight-for-age Z-score compared to those with the TT and TC genotypes ($P<0.05$). When examining individually in the case group and the control group, among these characteristics, only height in the cases was the index that differed between the three genotypes. Children with the CC genotype had the highest height, while the TT genotype had the lowest height ($P=0.02$). In the control group, no differences were observed in any other anthropometric indices, including weight, BMI, weight-for-age Z-score, height-for-age Z-score, BMI-for-age Z-score, and weight-for-height Z-score ($P>0.05$).

The results of testing the relationship between the *ADRB3*-rs4994 variant and obesity in different genetic models are shown in Table 3. Association of rs4994 with obesity was found in the dominant model, co-dominant model and recessive model. Using the TT genotype as the reference, statistical analysis indicated that the TC and CC genotypes increased the risk of obesity by 1.36 times ($P=0.05$) in the dominant model, the CC genotype increased the risk of obesity by 2.35 times ($P=0.024$) in the co-dominant model. In the recessive model, the CC genotype was associated with a 2.2-fold higher risk of obesity than the TC and TT genotypes ($P=0.039$).

The most suitable inheritance model for the *ADRB3*-rs4994 variant with obesity was evaluated by AIC values for all possible genetic models, and results showed that the co-dominant model was the best-fit model with the

Table 1 Characteristics of the study subjects

Parameter	Controls (n = 708)	Cases (n = 354)	*P*
Male (n, %)	532 (75.1)	266 (75.1)	1
Age (months)	59.5 (51.6 – 64.6)	59.8 (54.0 – 64.8)	0.408[b]
Weight (kg)	16.4 (14.6 – 18.3)	24.2 (22.2 – 26.9)	0.518[b]
Height (cm)	106.7 ± 7.2	110.2 ± 7.9	**< 0.0001**[a]
BMI (kg/m^2)	15.4 (14.6 – 16.5)	21.4 (20.9 – 22.6)	**< 0.0001**[b]
Height-for-age Z-score	-0.42 (-1.06 – 0.26)	0.48 (-0.56 – 0.91)	**< 0.0001**[b]
Weight-for-age Z-score	-0.23 (-0.84 – 0.56)	2.71 (2.19 – 3.28)	**< 0.0001**[b]
Weight-for-height Z-score	0 (-0.7 – 0.82)	3.60 (3.3 – 4.16)	**< 0.0001**[b]
BMI-for-age Z-score	-0.06 (-0.68 – 0.63)	3.35 (2.77 – 3.91)	**< 0.0001**[b]
Genotype frequency			
+ TT (n, %)	555 (78.4)	253 (71.5)	**0.016**
+ TC (n, %)	139 (19.6)	86 (24.3)	
+ CC (n, %)	14 (2.0)	15 (4.2)	
Allele frequency			
+ T (n, %)	1249 (88.2)	592 (83.6)	**0.003**
+ C (n, %)	167 (11.7)	116 (16.4)	

BMI: body mass index

[a]Data are mean ± SD. [b]Data are median (interquartile range)

P obtained by Student T test or Mann-Whitney U test or Chi-square test

Bold values indicate significant difference between cases and controls

Table 2 The characteristics of obesity-related traits in total, controls and cases according to the *ADRB3-rs4994* genotypes in Vietnamese children

Total (*n* = 1062)				
Characteristics	**TT (*n* = 808)**	**TC (*n* = 225)**	**CC (*n* = 29)**	***P***
Birth weight (kg)	3.3 ± 0.4	3.3 ± 0.4	3.3 ± 0.3	0.56[a]
Age (months)	57.3 ± 10.5	57.5 ± 9.8	58.1 ± 9.3	0.89[a]
Height (cm)	107.6 ± 7.5	108.3 ± 7.9	110.1 ± 8.9	0.13[a]
Weight (kg)	20.2 ± 5.0	20.9 ± 5.3	22.9 ± 6.5	**0.004**[a]
BMI (kg/m^2)	16.1 (14.8 – 20.0)	16.5 (14.9 – 20.3)	19.3 (15.6 – 21.1)	0.06[b]
Weight-for-age Z-score	0.35 (-0.62–2.05)	0.65 (-0.41–2.41)	2.03 (-0.16 – 3.25)	**0.03**[b]
Height-for-age Z-score	-0.19 (-0.86 – 0.58)	0.02 (-0.73 – 0.68)	-0.01 (-0.97 – 1.51)	0.09[b]
BMI-for-age Z-score	1.11 ± 1.87	1.33 ± 1.94	1.74 ± 1.85	0.07[a]
Weight-for-height Z-score	1.22 ± 1.93	1.51 ± 1.94	1.38 ± 1.99	0.36[a]
Controls (*n* = 708)				
Characteristics	**TT (*n* = 555)**	**TC (*n* = 139)**	**CC (*n* = 14)**	***P***
Birth weight (kg)	3.2 ± 0.4	3.2 ± 0.44	3.3 ± 0.38	0.84[a]
Age (months)	57.3 ± 10.6	56.6 ± 10.1	54.5 ± 8.9	0.16[a]
Height (cm)	106.7 ± 7.3	106.8 ± 6.9	105.5 ± 7.6	0.64[a]
Weight (kg)	17.6 ± 2.9	17.8 ± 3.1	17.8 ± 4.5	0.77[a]
BMI (kg/m^2)	15.2 (14.4 – 16.2)	15.2 (14.4 – 16.2)	15.6 (14.4 – 16.2)	0.87[b]
Weight-for-age Z-score	-0.22 (-0.89 – 0.43)	-0.28 (-0.73 – 0.44)	-0.16 (-1.16 – 0.16)	0.92[b]
Height-for-age Z-score	-0.42 (-1.01 – 0.27)	-0.36 (-0.90 – 0.27)	-0.61 (-1.07 – -0.03)	0.60[b]
BMI-for-age Z-score	0.03 ± 0.99	0.07 ± 1.12	0.18 ± 1.3	0.88[a]
Weight-for-height Z-score	0.10 ± 1.08	0.37 ± 1.20	0.35 ± 1.25	0.87[a]
Cases (*n* = 354)				
Characteristics	**TT (*n* = 253)**	**TC (*n* = 86)**	**CC (*n* = 15)**	***P***
Birth weight (kg)	3.4 ± 0.4	3.3 ± 0.4	3.4 ± 0.36	0.91[a]
Age (months)	57.2 ± 10.2	59.0 ± 9.3	61.4 ± 8.8	0.08[a]
Height (cm)	109.7 ± 7.7	110.8 ± 8.7	114.4 ± 8.1	**0.02**[a]
Weight (kg)	25.8 ± 3.9	26.0 ± 3.9	27.8 ± 3.9	0.07[a]
BMI (kg/m^2)	21.0 (20.2 – 22.0)	21.0 (19.5 – 22.1)	21.0 (19.8 – 22.6)	0.54[b]
Weight-for-age Z-score	2.55 (1.89 – 3.26)	2.44 (1.90 – 2.98)	3.00 (2.12 – 3.46)	0.36[b]
Height-for-age Z-score	0.47 (-0.35 – 1.08)	0.45 (-0.22 – 1.04)	1.20 (0.66 – 1.92)	0.09[b]
BMI-for-age Z-score	3.48 ± 0.89	3.38 ± 1.0	3.51 ± 0.72	0.40[a]
Weight-for-height Z-score	3.73 ± 0.57	3.84 ± 0.64	3.94 ± 0.51	0.53[a]

[a]Variables that are according to the standard distribution are represented by the mean ± standard deviation, *P* value obtained from the One-way ANOVA test

[b]Variables that are not according to standard distribution are represented by median and 25th – 75th percentile, *P* value obtained from the Krukall-Wallis test

lowest AIC values (AIC = 1349.90). For further multivariate analyses, we also used this model for analyzing the influence of SNP *ADRB3*-rs4994 on obesity in preschool children in Hanoi.

The combined effect of the *ADRB3*-rs4994 variant and eating behaviors (FR, EOE, EF, DD, SR, SE, EUE, FF) on the risk of obesity was examined by logistic regression because these eating behaviors were correlated with each other as well as with weight and BMI ($P < 0.01$) (Table 1S). As shown in Table 4, after adjustment for age, gender, living area, and eating behaviors, the odd ratios for CC and TC genotypes vs. TT reference genotype were still significantly associated with obesity.

Discussion

Results from our case-control study in Hanoi preschool children indicated that the *ADRB3*-rs4994 variant was strongly associated with obesity as an independent risk factor.

The replacement of tryptophan by arginine at position 64 in the ADRB3 protein affects the function of the β3-adrenergic receptor. This variant leads to reduced receptor sensitivity and impaired ability to stimulate lipolysis, which is the breakdown of fat in adipose tissue. As a result, individuals with the Trp64Arg variant have a decreased capacity for energy expenditure and fat burning. This can contribute to an increased risk of developing obesity and associated metabolic disorders [17, 24].

Table 3 Analysis of the best-fit model for the *ADRB3*-rs4994 variant for obesity

Models	Controls n (%)	Cases n (%)	OR (95% CI)	*P* value	AIC
Co-dominant					
TT	555 (78.4)	253 (71.5)	1		
TC	139 (19.6)	86 (24.3)	1.36 (1.01–1.85)	**0.050**	1349.90
CC	14 (2.0)	15 (4.2)	2.35 (1.12–4.94)	**0.024**	
Dominant					
TT	555 (78.4)	253 (71.5)	1		
TC + CC	153 (21.6)	101 (28.5)	1.45 (1.08–1.94)	**0.013**	1349.96
Recessive					
TT + TC	694 (98.0)	339 (95.8)	1		
CC	14 (2.0)	15 (4.2)	2.19 (1.05–4.60)	**0.037**	1351.73
Over-dominant					
TT + CC	569 (80.4)	268 (75.7)	1		
TC	139 (19.6)	86 (24.3)	1.31 (0.97 – 1 .78)	0.08	1352.91
Additive for copy number of C allele			1.45 (1.08–1.94)	**0.013**	1350.24

P value obtained by univariate logistic regression

Bold values indicate a statistically significant

95% CI: 95% Confidence interval, OR: odd ratio, AIC: Akaike's Information Criterion, BIC: Bayesian Information Criterion

Table 4 Association of the *ADRB3*-rs4994 variant with obesity in models considering children's eating behaviors

Models	Genotype	Obesity	
		OR (95%CI)	*P* value
Unadjusted	TT	1	
	TC	1.36 (1.01 – 1.85)	**0.050**
	CC	2.35 (1.12 – 4.94)	**0.024**
Adjusted for age, gender, residence, birth weight	TT	1	
	TC	1.37 (1.01 – 1.86)	**0.048**
	CC	2.36 (1.12 – 4.99)	**0.024**
Adjusted for age, gender, residence, birth weight, and FR	TT	1	
	TC	1.33 (1.01 – 1.87)	**0.049**
	CC	2.71 (1.2 – 6.08)	**0.016**
Adjusted for age, gender, residence, birth weight, and EOE	TT	1	
	TC	1.37 (1.03 – 1.88)	**0.048**
	CC	2.47 (1.16 – 5.24)	**0.019**
Adjusted for age, gender, residence, birth weight, and DD	TT	1	
	TC	1.38 (1.01 – 1.88)	**0.045**
	CC	2.49 (1.17 – 5.30)	**0.018**
Adjusted for age, gender, residence, birth weight, and SR	TT	1	
	TC	1.35 (1.01 – 1.90)	**0.047**
	CC	2.98 (1.22 – 7.27)	**0.017**
Adjusted for age, gender, residence, birth weight, and EUE	TT	1	
	TC	1.37 (1.01 – 1.89)	**0.049**
	CC	2.15 (1.01 – 4.95)	**0.046**
Adjusted for age, gender, residence, birth weight, and FF	TT	1	
	TC	1.37 (1.01 – 1.86)	**0.048**
	CC	2.38 (1.23 – 5.01)	**0.023**

P values obtained by multivariate logistic regression

Bold values indicate a statistically significant

95% CI: 95% Confidence interval, OR: odd ratio. FR: food responsiveness, EOE: emotional overeating, EF: enjoyment of food, DD: desire to drink, SR: satiety responsiveness, SE: slowness in eating, EUE: emotional undereating, FF: food fussiness

ADRB3-rs4994 variant was demonstrated to be associated with some health problems such as being overweight, abdominal obesity, HDL-C levels, harder to lose weight, a reduced basal metabolic rate, and type 2 diabetes in previous studies [17, 25–27]. Adipocytes with the TC and CC genotypes exhibited a weaker response to β_3-adrenergic agonists as their cytosolic cAMP and glycerol were nearly 70% lower compared with that of TT genotype [27, 28]. In addition, the existence of the C allele in genotypes also leads to a lower rate of enzyme activity for the lipolysis induced by *ADRB3* [29]. Thus, the replacement of T with C in codon 64 of the *ADRB3* gene significantly decreased the lipolysis rate in brown adipocytes.

Much research has been carried out to clarify the relationship between *ADRB3*-rs4994 and obesity in humans, but the outcomes are still controversial. *ADRB3*-rs4994 was demonstrated to be associated with obesity in many populations such as Chinese [30], France [31], and Japan [31]. A study involving 714 preschool children in Wuhan, China, revealed a significant difference in the *β3-AR* gene variant between girls with varying degrees of obesity and normal children. In the homozygous variant, elevated levels of triglycerides and decreased levels of high-density lipoprotein were observed, and these differences were statistically significant. The study concluded that the distribution of the Trp64Arg variant in the *ADRB3* gene among preschool children is associated with simple obesity [32]. A meta-analysis incorporating data from 16 studies, encompassing 5,147 cases of overweight/obesity and 7,350 non-obese controls, with a substantial proportion (69.9%) of the subjects were sourced from East Asia. In the overall population meta-analysis, statistically significant associations were identified, indicating an increased risk of childhood and adolescent overweight/obesity in the allele model (OR = 1.23, 95% CI = 1.10 – 1.38), heterozygote model (OR = 1.39, 95% CI = 1.16 – 1.68), and dominant model (OR = 1.31, 95% CI = 1.12 – 1.54). Further stratified analysis based on geographical regions revealed that statistical significance was observed exclusively in the East Asia subgroup for the allele model, homozygote model, heterozygote model, and dominant model. In summary, the meta-analysis indicates a significant association between the *ADRB3*-rs4994 variant and an elevated risk of childhood and adolescent with overweight/obesity, particularly within the East Asian population [23].

Conversely, some research indicated that there was no association of the *ADRB3*-rs4994 variant with obesity. According to research by Kurokawa et al. on 87 Japanese children, BMI and body fat percentage were not statistically different among the TC, CC, and TT genotypes

[33]. Research by Porto et al. (2004) on 934 high school students (121 normotensive and 54 hypertensive students) showed that there was no association between increasing BMI and rs4994 variant of the *ADRB3* gene. The frequencies of TT, TC, and CC genotypes were 85%, 14.5%, and 0.5%, respectively [34]. Research by Chou et al., on 559 adolescent volunteers in Taiwan, did not found any association between obesity and the rs4994 variant of *ADRB3* gene in Taiwanese adolescents. The frequencies of TT, TC, and CC genotypes were 72.3%, 26.1%, and 1.6%, respectively. And these genotypes were found to be in Hardy-Weinberg equilibrium [35].

A study by Yilmaz et al. (2019) conducted on 441 children and adolescents aged 6–18 years in Turkish also resulted in no association of *ADRB3*-rs4994 with obesity. However, the frequency of the rs4994 variant was higher in obese girls, which can lead to weight gain. The frequencies of the TT, TC, and CC genotypes in the obese group were 84.8%, 14.4%, and 0.8%, while in the control group, the frequencies of these genotypes were 89.4%, 10.6%, and 0.0%, respectively *(P*=0.247*)*. There was no significant difference in terms of allele and genotype frequencies between the two study groups. The frequencies of T and C alleles were 92.0% and 8.0% in the obese group, whereas the frequencies of these alleles were 94.7% and 5.3% *(P*=0.127*)* in the control group [36].

Generally, the inconsistencies among the previous studies' results may be due to differences in study populations (sex, age, socio-economic status, etc.), and environmental or lifestyle factors (levels of energy intake and level of physical activity). In addition, the allele frequency at locus rs4994 varies in different ethnic populations. With a frequency of 0.38 in Eskimos and 0.31 in Indians, the C allele was very common in both populations [37]. Japanese population had a high C allele frequency of 0.21, which is twice as common as in white populations [38]. In our study, the C allele accounted for 0.12 in the controls and 0.16 in the cases.

Along with the development of social life, Vietnam has experienced a sharp rise in the prevalence of overweight and obesity in recent years, particularly in children. This phenomenon may be caused by the gradual replacement of healthy foods with various high-calorie but low-nutrient foods and beverages, which leads to a substantial change in the eating behaviors of Vietnamese children, especially those living in big cities. When emotions change, students often have no control over the amount of food they eat. According to Derks et al. (2018), EOE has a two-way relationship with BMI, both as a predictor and as a result of high BMI [39]. Ashcroft et al. (2008) also showed that emotional overeating increased over time from 4 years of age to 10 years of age [40]. In addition, a study of twins in the UK showed that overeating in response to negative emotions was a learned behavior rather than a genetic factor [41]. In addition, food responses (such as demanding food, if allowed, students can eat a lot, when they are full, they can still eat…) were also one of the risk factors for increasing BFP. A longitudinal study of 3,331 children in the Netherlands showed that, by the age of 4, food response index (FR) and food preference (EF) were higher in children with high BMI. When children were 10 years old, there was a positive relationship between food response index (FR), food preference (EF) and mood swings (EOE) with BMI and body fat mass. Meanwhile, satiety response (SR) was negatively associated with BMI and body fat mass [39]. Therefore, the association of the *ADRB3*-rs4994 variant with childhood obesity was adjusted for children's eating behaviors.

Nutrigenomics is a new field of study whose objective is to clarify the relationship between the genetic factors of an individual and the corresponding dietary intake, and also the effect of nutrition on gene expression. Based on the PubMed database, Pavlidis et al. (2015) conducted a meta-analysis to examine the association of 38 candidate genes with dietary intake and/or pathologies of nutrient-related diseases. Although his results showed that there was no significant association in any of these 38 genes, it is clear that additional research on nutrigenomics needs to be carried out because this is a potential tool that provides many benefits for medicine [42].

Since our result indicated that there was a significant association between the *ADRB3*-rs4994 variant with obesity regardless of children's eating behaviors, it may be useful for doctors, professional nutritionists and other healthcare professionals to categorize children into groups with different risks of obesity, thus providing suggestions for their parents about specific diets or exercise routines to prevent obesity. Despite this, our cross-sectional study also had certain disadvantages due to not considering the contribution of lifestyle factors and their complicated interaction with other genetic factors to the development of childhood obesity. Furthermore, although the CEBQ has been tested in various groups of children, it has not been formally validated in the Vietnamese children population through published studies. Therefore, further research needs to be conducted in the future to examine the roles of other factors in the epidemiology of obesity.

Conclusions

In summary, there was an association between the rs4994 variant of the *ADRB3* gene and obesity in Vietnamese preschool children, independently of their eating behaviors. As obesity is preventable, it is very important to consider applying intervention through genotypic risk assessment to prevent and counteract obesity and its complications.

Methods

Study populations

The present study enrolled 1,062 3-5-year-old unrelated Vietnamese children from 36 kindergartens in Hanoi, Vietnam. Our study excluded all children with psychological issues, atherosclerosis, heart disease, diabetes mellitus, endocrine disorders, infectious diseases, and HIV/AIDS.

From 2019 to 2020, a case-control study was performed on 354 children with obesity and 708 children with normal weight recruited using WHO (2006) criteria for children under 5 years old. The sample size for a case-control study involving a qualitative variable was calculated [43] with a control-to-case ratio of 2:1. Based on a previous study [44], the estimated proportion of exposure among cases was 0.3, and among controls was 0.2. A standard normal variate of 1.28 was used to achieve 90% power, and the standard normal variate corresponding to a 95% confidence level was 1.96. Consequently, the study required a minimum of 296 children in the case group and 592 children in the control group.

Children were classified as obese if their weight/height Z-score > +3SD for children under 5 years old or BMI Z-score by age and sex > +2SD for children upper 5 years old. To exclude children asymptotically malnourished or overweight, children were considered normal weight as -1SD ≤ weight/height Z-score ≤ +1SD for children under 5 years old or -1SD ≤ BMI Z-score ≤ Mean for children upper 5 years old.

Ethics statement

The parents or guardians of all children were clearly explained the purposes of the study. They were given and signed the written informed consent form. The personal information was kept strictly confidential, and the data was used only for research purposes. All participants had the right to quit the study whenever they wanted. Medical Ethics Council of the Institute of Nutrition with Decision No. 343/VDD-QLKH on July 27, 2018.

Measurements

Children's anthropometric measurements, including weight, and height were taken twice following the Loman standard method [45], and the average was used for analysis. Children were dressed simply in lightweight clothes and without shoes when they were taken their anthropometric indices. Using standardized medical scales, the body weight was calculated to the nearest 0.1 kg and the measurement of height was taken down to the nearest 0.1 cm. BMI indices were determined by the weight per square of height (kg/m^2).

Assessment of eating behavior

Children's Eating Behavior Questionnaire (CEBQ) was used in this study. This is a set of questions evaluated by experts with high reliability and consistency [46]. Parents or guardians choose the child's eating habits according to how often they occur (never, rarely, sometimes, often, always). The questions that assess the current 8 factors of children's eating behavior are divided into 2 groups [47]: (1) Group 1: food approach includes the variables food responsiveness (FR), emotional overeating (EOE), enjoyment of food (EF) and desire to drink (DD); (2) Group 2: food avoidance includes the variables satiety responsiveness (SR), slowness in eating (SE), emotional undereating (EUE) and food fussiness (FF). Each item was rated on a 5-point Likert scale from 1 (never) to 5 (always), with higher scores indicating stronger expressions of the respective eating behaviors. Subscale scores were computed by averaging the scores of items within each subscale.

DNA extraction

DNA was extracted from the sample of the cheek mucosa cell using the GeneJET Genomic DNA Purification kit (Thermo, USA) according to the manufacturer's instructions. All the DNA samples had an A260/A280 ratio ranging from 1.8 to 2 to ensure the purification of the study.

Genotyping method

The genotype of each child at the *ADRB3*-rs4994 locus was identified by the polymerase chain reaction-restriction fragment length polymorphism method (PCR–RFLP). The sequence of the primers used for the PCR reaction was designed by the research group. The forward primers had the following sequence 5'- CGCCCA ATACCGCCAACAC-3', and the sequence of the reverse primers was 5'-CCACCAGGAGTCCCATCACC-3' [48]. The PCR mixture was initially heated to 94 °C for 3 min, then 34 reaction cycles were repeated as follows: denaturation at 94 °C in 30 s, lowering the temperature to 64 °C for annealing in 30 s, raising the temperature to 72 °C for extension in 30 s. The extension stage of the last cycle was performed in 8 min, and the products were kept cold at 4 °C.

The amplified DNA segments with a length of 210 bp then were incubated with Fast Digest *Mval* restriction enzyme (Thermo Fisher Scientific, USA) at 37 °C for 15 min. Products of restriction enzyme digestion were separated by gel electrophoresis on Redsafe-stained 2.5% agarose gel at 100 V. The distribution of restriction fragments on the agarose gel was observed by UV illumination, and these results can be used to distinguish different genotypes at the *ADRB3*-rs4994 locus. The band patterns of CC, TC, and TT genotypes were (158 bp + 31 bp + 15

bp+6 bp), (158 bp+31 bp+15 bp+6 bp), (97 bp+61 bp +31 bp+15 bp+6 bp), respectively, but DNA fragments with the length of 31 bp, 15 bp, and 6 bp could not be observed on the agarose gel due to their small sizes that made them move out of the gel plate.

The genotyping results from PCR–RFLP method were checked for accuracy by comparing them with genotyping results from dideoxy chain termination sequencing. 10% of the samples were randomly selected to identify their genotypes using the BigDye® Terminator v3.1 cycle sequencing chemistry kit (Axil Scientific Pte Ltd, Singapore). The results from the two methods above were identical, these indicated that the designed method can precisely identify genotypes of the rs4994 variant.

Statistical analysis

The obtained data were statistically analyzed using SPSS 23.0 software. Measurements of study subjects were represented as percentages (%) for the qualitative variables. For quantitative variables, the characteristics were shown in the form of mean ± standard deviation if they were in the normal distribution, or were expressed in median and 25th – 75th percentiles in case of other types of distribution. Significant differences between data groups were identified by either χ^2 tests, the Kruskall-Wallis test, Student t-test, Fisher Exact test, Man-Whitney-U-test, or ANOVA in each specific comparison. Hardy-Weinberg equilibrium of the studied population was checked by Fisher's exact test. The inheritance mechanism of alleles at the *ADRB3* rs4994 locus was tested in five genetic models including dominant, recessive, co-dominant, additive and over-dominant models. SNPstats software was used to choose the most suitable genetic model following Akaike's Information Criterion (AIC). Binary logistic regression analysis was used to examine the association between the rs4994 variant and obesity in preschool children, and the results were given as odds ratios (OR) with 95% Confidence intervals (CI). For the statistical analysis, 8 scales of CEBQ (FR, EF, DD, SR, SE, FF, EUE, EOE), age, sex, and living area were adjusted. Differences were considered as statistical significance if the $P<0.05$.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-05073-7.

Supplementary Material 1

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Author contributions
NTHH performed the experiments, analyzed the data, and wrote the manuscript; NTTT, and DTNT analyzed the data, discussed and edited the final draft for publication; LTT, and NTTT collected the data, participated in manuscript draft preparation; LTT designed the research, analyzed the data, and edited of the final draft for publication.

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Data availability
Supplementary file data.

Declarations

Ethics approval and consent to participate
The project was approved by the Medical Ethics Council of the Institute of Nutrition with Decision No. 343/VDD-QLKH on July 27, 2018. All methods were carried out in accordance with relevant guidelines and regulations. The parents or guardians of all children were clearly explained the purposes of the study. They were given and signed the written informed consent form.

Consent to publish
NA.

Conflict of interest
All author declares that there is no conflict of interest.

Competing interests
The authors declare no competing interests.

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