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Body Adiposity Changes After Lifestyle Interventions in Children/Adolescents and the *NYD-SP18* and *TMEM18* Variants

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABDF 1 **Lukas Zlatohlavek**
ABE 2 **Vit Maratka**
ABD 1 **Eva Tumova**
ADE 1 **Richard Ceska**
CDE 3 **Vera Lanska**
ACDEF 1 **Michal Vrablik**
ACDEFG 3 **Jaroslav A. Hubacek**

1 3rd Department of Medicine, 1st Faculty of Medicine of Charles University and General University Hospital in Prague, Prague, Czech Republic
2 Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
3 Dr. Filip's Institute for Children, Poděbrady, Czech Republic

Corresponding Author: Lukas Zlatohlavek, e-mail: lukas.zlatohlavek@lf1.cuni.cz
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Background: This study was carried out to determine the relationship between the common *TMEM-18* (rs4854344, G>T) and *NYD-SP18* (rs6971091, G>A) gene variants and weight loss after lifestyle interventions (increased physical activity in conjunction with optimal dietary intake) in overweight/obese children/adolescents.

Material/Methods: We genotyped 684 unrelated, white, non-diabetic children (age 12.7±2.1 years, average BMI at baseline 30.66±4.80 kg/m^2). Anthropometric and biochemical examinations were performed before and after 4 weeks of an intensive lifestyle intervention.

Results: The mean weight loss achieved was 5.20±2.02 kg (P<0.001). *NYDSP-18* AA homozygotes had significantly higher abdominal skinfold value before and after the intervention (both, P=0.001). No significant associations between BMI decrease and the *NYD-SP18* and *TMEM18* variants were found. Associations between all anthropometrical and biochemical changes and genes remained non-significant after data were adjusted for sex, age, and baseline values.

Conclusions: Decreased body weight in overweight/obese children is not significantly influenced by the *NYD-SP18* rs6971091 or *TMEM18* rs4854344 polymorphisms.

MeSH Keywords: **Child • Intervention Studies • Obesity • Polymorphism, Single Nucleotide**

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Background

Obesity is one of the common risk factors of type 2 diabetes mellitus and cardiovascular diseases. Obesity/overweight is primarily the result of a positive energy balance, which is largely caused by the combination of excess energy intake and insufficient physical activity. However, other frequently omitted and less discussed factors, such as the adverse effects of many commonly used drugs, sleep deficit, and excess thermal comfort, also play a significant role [1,2].

Thus, BMI (body mass index) is significantly influenced by both environmental and genetic factors. Studies of twins estimate that as much as 60% of body mass index/body weight variability is attributable to genetics. Similarly, the response to interventions focused on body weight loss falls under a certain degree of genetic control [3].

A polymorphism in the vicinity of the *NYD-SP18* gene was identified using fine mapping of the chromosome 7 region, which is known for its high obesity LOD-score. Both the NHLBI Family Heart Study and the Framingham Study report that the effect of the *NYD-SP18* rs6971091 polymorphism on BMI values is even greater [4] than that of the 1st intron tagging *FTO* variants that have a well-established effect on body weight [5]. These results were partially confirmed by the Czech Post-MONICA study, in which an association between the *NYD-SP18* polymorphism and BMI was detected in males but not in females [6]. Results from the large HAPIEE study of elderly subjects detected no significant association between the *NYD-SP18* polymorphism and BMI [7].

An original study [4] reported that the *NYD-SP18* polymorphism may influence plasma leptin levels. *NYD-SP18* is also associated with the production of testosterone and, therefore, may affect exercise-based muscle formation and development, which may be more pronounced in males. This finding potentially explains the sex-specific effect of *NYD-SP18* on BMI observed in the Czech Post-MONICA study, which included males age 25–64, and the HAPIEE study, which included males aged over 45 years [7]. Finally, it has been suggested that this variant is a significant predictor of body composition changes in overweight/obese females after a short-term lifestyle intervention [8].

Additionally, the intronic variant rs4854344 within the *TMEM18* gene is, based on the results of a GWA (genome-wide association) study, a very strong predictor of BMI/body weight in whites [9]. Original results have also been replicated in children/adolescents [3,10]. The exact functional link between the transmembrane TMEM18 protein and BMI is not clear, but animal model research suggests that TMEM18 could be a significant regulator of food intake and energy expenditure [11]. The few studies to have examined the possible effect of this gene in determining the success of interventions leading to body weight loss in children/adolescents have produced conflicting results [12,13].

The aim of this large study was to assess how the *NYD-SP18* and *TMEM18* variants affect the efficacy of weight loss interventions in children/adolescents.

Material and Methods

Analyzed individuals

A total of 684 individuals (280 boys and 404 girls) recruited between May 2010 and August 2015 (age 12.7±2.1 years) agreed to participate in the study. A complete data set (biochemical and anthropometric parameters) along with DNA samples were available in all cases. The exclusion criteria were: diagnosed diabetes mellitus (both types), smoking, arterial hypertension, contraindication of prescribed physical activity and, finally, the use of medications. All of the participants were white. The protocol of the study was published previously [14,15] and is briefly summarized below.

Intervention

The participants were enrolled in an intensive 1-month inpatient weight reduction program. The program comprised individualized dietary changes aimed at achieving a caloric intake of 5000 kJ for children aged 8–10 years old and 7000 kJ for adolescents 11–15 years old. All of the participants were assessed by an exercise specialist. Children performed 5 units of supervised physical activity daily, totalling at least 120 minutes of endurance training (heart rate 65–75% of maximum). The exercise program consisted of aerobic and resistance training complemented by playing ball games, swimming, and brisk walking [13,15].

Measurements

For the anthropometric measurements, all patients removed their shoes and wore only underwear. Body weight was measured with a horizontally placed and calibrated electronic weight scale (scaled to the nearest 100 g). The height, waist circumference (defined as the narrowest diameter between the xiphoid process and iliac crest), and hip circumference (defined as the widest diameter over the greater trochanters) of each child were measured to an accuracy of 0.5 cm (without clothing). BMI (kg/m^2) was calculated from the obtained measurements. Skinfold thickness was measured using a BEST II K-501 caliper. The measurements were performed in 3 areas: the subscapular area (in the projection of the angle of the left scapula), the triceps area (in the middle between the acromion and the elbow), and the abdominal area (1/3 of the distance

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Table 1. Basic characteristics of the participants before and after intervention.

	Before intervention	After intervention	P
Age (years)	12.71±2.06	–	
N (males/females)	684 (280/404)	–	
Body height (cm)	161.05±11.35	–	
BMI (kg/m^2)	30.66±4.80	28.54±4.53	0.001
Body weight (kg)	79.7±19.6	74.2±18.4	0.001
Total cholesterol (mmol/L)	4.33±0.92	3.43±0.74	0.001
LDL-cholesterol (mmol/L)	2.65±0.76	1.99±0.62	0.001
HDL-cholesterol (mmol/L)	1.23±0.26	1.09±0.62	0.001
Triglyceridemia (mmol/L)	0.98±0.48	0.76±0.29	0.005
Glycaemia (mmol/L)	4.93±0.41	4.88±0.46	0.05
Waist (cm)	89.01±11.05	83.26±10.32	0.001
Hip (cm)	103.77±11.89	98.45±11.45	0.001
Abdominal skinfold (cm)	4.36±1.06	3.67±0.94	0.001

between the navel and the left anterior superior iliac spine). All of the measurements were performed by a single experienced nurse to eliminate inter-individual variability.

DNA analysis

Genomic DNA was extracted from the peripheral white blood cells of patients. Both polymorphisms were genotyped using PCR-RFLP. Amplifications were run in a total volume of 25 µL, containing 100–300 ng of genomic DNA, 1× standard PCR buffer without $MgCl_2$, 1.5 mmol/L $MgCl_2$, 10 pmol of each primer, 200 µmol/L of each dNTP, and 0.5 U of Taq DNA polymerase. The mixture for the analysis of the *TMEM18* genotypes included a further 6% of DMSO.

The DNA fragment containing the NYD-SP18 SNP rs6971091 polymorphism was amplified using the oligonucleotides 5' cct tgg tca tta gct gaa tga gaa gct and 5' aag gcc tta acc tgg ttc tgc [6] according the following conditions: the initial denaturation was 96°C for 3 min, followed by 34 cycles at 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. The last extension was prolonged to 3 min. The PCR product (105 bp) was cut with 5 units of the HindIII restriction enzyme. Restriction fragments of 79 bp and 26 bp represented the A allele, whereas the presence of an uncut product represented the more common G allele.

For the genotyping of the rs4854344 variant within the *TMEM18* gene, oligonucleotides 5' atg cat tgt tag gca att ttg tca ttg tgc and 5' tta gat aca cac tct cca ctg tgt tag agc were used [16]. PCR conditions were as follows: the initial denaturation was 95°C for 3 min, followed by 34 cycles at 95°C for 15 s, 66°C for 30 s, and 72°C for 30 s. The last extension was prolonged to 3 min. The PCR product (199 bp) was cut with 5 units of the SacI restriction enzyme. Restriction fragments of 167bp and 32bp represented the G allele, whereas the presence of an uncut product represented the major T allele.

The chemicals used were purchased from Fermentas International, Inc. (Burlington, Ontario, Canada). PCR reactions were performed using the DYAD Disciple PCR instrument from MJ Research (Waltham, MA, USA).

Statistical analysis

Deviations from Hardy-Weinberg equilibrium were tested using the tools at *http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls*. The paired *t* test was used for comparison of before and after intervention. Highly skewed variables were log-transformed before the analysis. The *t* test, ANOVA, and ANCOVA (to adjust for age, sex, and baseline values of each individual variable) were used for the comparison of independent groups. In the case of the *TMEM18* variant, as there were less than 2% of minor homozygotes, these were pooled with the heterozygotes for analysis purposes. All of the tests were 2-tailed with p values less than 0.05 considered significant.

Results

A positive change was observed in the anthropometric parameters of interest after the intervention. There was a highly significant decrease in average BMI and waist circumference, suggesting a reduction in visceral fat mass (Table 1).

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Table 2. Characteristics of the study participants before (**A**) and after (**B**) intervention (mean ±SD) by *NYD-SP18* rs6971091 genotype.

(A)	*NYD-SP18* rs6971091			P
	GG	GA	AA	
N	353	272	54	
BMI (kg/m²)	30.76±4.90	30.40±4.57	31.34±5.28	0.42
Hip (cm)	104.1±11.8	103.2±11.5	105.1±14.1	0.40
Waist (cm)	89.7±10.8	88.0±11.0	90.0±12.7	0.15
Abdominal skinfold (cm)	4.39±1.06	4.25±1.09	4.81±1.09	0.001
Weight (kg)	80.34±19.42	78.64±19.38	81.53±22.16	0.44
(B)	***NYD-SP18* rs6971091**			**P**
	GG	**GA**	**AA**	
N	353	272	54	
BMI (kg/m²)	28.62±4.61	28.34±4.33	29.11±5.06	0.48
Hip (cm)	98.8±11.5	97.8±10.9	99.8±13.4	0.36
Waist (cm)	83.8±10.1	82.3±10.0	84.6±12.7	0.11
Abdominal skinfold (cm)	3.70±0.93	3.58±0.85	4.14±0.96	0.001
Weight (kg)	74.84±18.24	73.20±18.04	75.78±20.78	0.45

Table 3. Characteristics of the study participants before (**A**) and after (**B**) intervention (mean ±SD) by *TMEM18* rs4854344 genotype.

(A)	TMEM18 rs4854344		P
	GG	+T	
N	508	173	
BMI (kg/m²)	30.65±4.78	30.67±4.81	0.98
Hip (cm)	104.1±12.0	102.8±11.5	0.31
Waist (cm)	89.1±10.9	86.7±11.2	0.65
Abdominal skinfold (cm)	4.37±1.08	4.36±1.11	0.74
Weight (kg)	79.96±19.74	79.25±19.36	0.67
(B)	***TMEM18 rs4854344***		**P**
	GG	**+T**	
N	508	173	
BMI (kg/m²)	28.54±4.55	28.54±4.46	0.99
Hip (cm)	98.6±11.7	97.8±10.5	0.42
Waist (cm)	83.3±10.3	82.9±10.2	0.65
Abdominal skinfold (cm)	3.69±0.90	3.65±0.97	0.63
Weight (kg)	74.43±18.49	73.70±18.06	0.65

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High inter-individual diversity in response to the intensive short-term intervention was observed; the highest body weight decrease was 14.4 kg and the lowest was only 0.1 kg.

The call rates of the *NYD-SP18* rs6971091 and *TMEM18* rs4854344 variants were 99.3% and 99.1%, respectively. The genotype frequencies were 52.0% (GG), 40.0% (GA), and 8.0% (AA) for the *NYD-SP18* polymorphism and 73.7% (TT), 24.7% (TG), and 1.6% (GG) for the *TMEM18* polymorphism, which are similar to the frequencies obtained for the general Czech adult population [5,6]. The Hardy-Weinberg test confirmed the independent segregation of individual genotypes (P=0.97 and P=0.42).

Baseline measurements showed only 1 significant difference within the analyzed group: *NYDSP-18* AA homozygotes had significantly higher abdominal skinfold value, both before and after the intervention (P<0.001) (Tables 2, 3).

Most importantly, neither the *NYD-SP18* rs6971091 variant nor the *TMEM18* rs4854344 variant was a significant determinant of successful intervention. Changes in body weight, BMI, and abdominal skinfold did not differ significantly between the individual genotypes (Tables 2, 3). Finally, changes in biochemical parameters were not associated with the analyzed polymorphisms. The results remained non-significant after adjusting for baseline values of the analyzed parameters, age, and sex.

Discussion

The study presented here is the first to focus on the potential role of the *NYD-SP18* gene polymorphism in determining weight loss and/or changes in body composition after an intensive lifestyle intervention (5 units of physical activity per day under close supervision in an in-patient facility simultaneously with a change in dietary habits in respect of sex/age-matched optimum values) in children/adolescents. Further, the *TMEM18* variant was also analyzed. Neither *NYD-SP18* nor the *TMEM18* variant was associated with changes in anthropometric measurements after the lifestyle intervention.

To date, the putative association between the *NYD-SP18* rs6971091 polymorphism and BMI/body weight has been reported in 2 [4,6] out of 3 previous studies [4,6,7]. Although we did not find an association of this polymorphism with BMI values in our study, one of the obesity markers – abdominal skinfolds value – was significantly higher in AA homozygotes both before and after the intervention.

One published study of overweight/obesity treatment efficacy in healthy adult females [8] reported that GG homozygotes profit more from lifestyle interventions than A allele carriers. In contrast, we did not detect a significant effect of this SNP on intervention success. Although this is not so surprising in boys, we also did not detect a significant effect in girls. The differences between the results of the studies may also have been caused by the different parameters examined and different interventional protocol used. For instance, in contrast to Suchanek et al. [8], who focused on changes in body fat mass and body muscle mass, we compared body weight, body mass index, and abdominal skinfold.

Few studies on similar topics, including *TMEM18* polymorphism, have been published [12,13,17]. Similar to our results, Hiney et al. [17] analyzed 282 obese children and found no evidence for effects of this gene on weight loss or weight regain at 1 year after an intervention, and similar results were observed in an analysis of 400 children/adolescents [13]. However, a Spanish study detected a slight effect on decrease of fat mass in 168 overweight/obese adolescents [12], and gene score (which includes *TMEM18* polymorphism) was a significant predictor of intervention success.

Overall, it seems that the effects of the *TMEM18* polymorphisms *per se* are not strong enough to effect body composition/body changes after an intervention in children/adolescents. *TMEM18* could potentially be a part of the genetic scoring system for predicting the success of such interventions.

Generally, directly comparing intervention studies focused on body weight loss is very difficult, principally due to the different study protocols used. For example, the variability of dietary changes can be based not only on prescribed recommendations but also on subject compliance [18]. Our type of study, in which patients are virtually isolated and given an identical menu, minimizes the intra-study variability caused by different energy intake values. Also, the intensity and frequency of physical activity performed was highly variable in other studies, but uniform in our study. The fact that both these factors were strictly controlled in our study is undoubtedly a strong point. Unfortunately, however, such a strict regime cannot be sustained over the long term. Finally, the pre-selection of participants and the relatively low number of subjects enrolled (frequently less than 100 individuals) means that such interventional studies are prone to either false-positive or false-negative results. Our study included almost 700 children/adolescents and is therefore one of the largest performed thus far.

Intervention studies have tested dozens of genes in an attempt to uncover a genetic principle that contributes to differences in post-intervention weight loss. Unfortunately, based on the above evidence, it is not surprising that these studies have delivered rather inconclusive results, the replications of which are problematic [3,9,10,12,14,15,17,19–25].

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Conclusions

We conclude that the rs6971091 *NYD-SP18* and rs4854344 *TMEM18* variants are most likely not major genetic determinants of BMI decrease after short but intensive lifestyle interventions in children/adolescents. Other genes need to be analyzed to detect powerful genetic determinants with potential to predict obesity treatment success and to tailor an individual intervention plan.

Compliance with ethical standards

All procedures performed involving human participants were in accordance with the ethical standards of the respective institutional and/or national research committees and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This article does not contain any studies involving the use of animals. Informed consent was obtained from parents/guardians of all individual participants enrolled in the study.

Conflict of interest

None.

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