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ETHNIC DIFFERENCE FREQUENCIES OF THSD7A VARIANT, BUT NO ASSOCIATION WITH OBESITY

ABSTRACT: The THSD7A *gene has recently been described as a "obesity gene". The product of this gene significantly modulates angiogenesis, a process linked to adipogenesis, growth of adipose tissue and the development of an obese phenotype. It can therefore be assumed that the role of this gene in obesity may be due to its angiogenic activity. The aim of this study was to analyze allele and genotypic frequencies of rs1526538 polymorphism in* THSD7A *gene and investigate its possible relationship with obesity indicators. The study included 407 individuals of Roma origin and 229 individuals of the majority population in eastern Slovakia. Anthropometric measurements of body height (cm), body weight (kg), waist circumference (cm), and hip circumference (cm) were performed by standard methods to all participants, and BMI, WHR and WHTR indexes were calculated. Buccal mucosa sample were obtained from all the participants and DNA isolation was performed by the commercial kit. SNP genotyping was done using the TaqMan SNP Genotyping Assays. The frequency of the risk allele A was 47.9% in the Roma population and 60.9% in the Slovak majority, suggesting ethnic variability in the frequency of this polymorphism. No significant relationship of this polymorphism with obesity was confirmed in association analysis. Our study provided the evidence for ethnic difference frequency of rs1526538 in* THSD7A *gene, but did not confirm relationship between this polymorphism and obesity traits. However, further studies are needed to confirm or reject the hypothesis that this genetic determinant of angiogenesis is involved in the etiopathogenesis of obesity.*

KEY WORDS: THSD7A gene – Obesity – Polymorphism – Association study – Angiogenesis

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INTRODUCTION

Obesity results from an imbalance between energy income and expenditure. As a result, adipose tissue growth and pathological changes occur. Obesity is a common, chronic disease and cardiometabolic risk factor, but it increases the prevalence of many complications associated with multiple diseases (Sutherland 2014, Corvera, Gealekman 2014, Jokinen 2015, Engin 2017). A multifactorial disease such as obesity is undeniably of complex origin. It is the result of the interplay of several external factors (stress, nutrition, smoking, physical activity, infection, etc.) and a complex genetic contribution. However, the inheritance of a common form of obesity is not so simple. Many genes at different loci are involved in the formation of a common form of obesity, with each gene having little effect on the phenotype.

The massive development of molecular genetics has made it possible to research candidate genes for obesity through genetic association analysis. In recent years, a large number of genes have been identified whose particular variants associate with a higher risk of obesity. The candidate VEGFA gene, which is a pro-angiogenic factor, is located on the sixth chromosome. On the third chromosome, ADAMTS is an important angioinhibiting factor. Other candidate genes affecting the angiogenic environment (LEP, LEPR, TNF, TGFB1, IL6, etc.) can also be found in the human obesity gene map. Therefore, it can be assumed that genes affecting angiogenesis could be predeterminants for obesity.

One of the latest "obesity genes" is the THSD7A (Nizamuddin *et al.* 2015), whose polymorphic variant rs1526538 in the intron region is associated with higher BMI and thus obesity in the Indian population. The risk allele is A variant, the ancestral is the G allele. Cytogenetic gene localization is at locus 7p21.3 (GeneCards 2020). In vitro studies have shown that the THSD7A gene product modulates angiogenesis (Wang *et al.* 2010, 2011, Kuo *et al.* 2011). The process of angiogenesis is closely linked to adipogenesis, adipose tissue growth and consequently the development of obese phenotype (Lijnen 2008, Cao 2010, Lemoine *et al.* 2013, Corvera, Gealekman 2014) and it can therefore be assumed that the gene's influence on obesity development is via its angiogenic function.

The Roma population is a genetically isolated group inhabiting mainly the European continent. Its origin is in northeast India, where it began to migrate to the west (Ginter *et al.* 2001). It is estimated that Slovakia is among the countries with the highest number of Roma individuals in Europe (Bernasovsky, Bernasovska 1999). The Roma ethnicity has its health, cultural but also genetic specifics and therefore represents a unique source of information for anthropogenetic research. The Roma are characterized by a high prevalence of obesity (Vozarova De Courten *et al.* 2003, Dolinská *et al.* 2007, Mačeková *et al.* 2010, Petrašová *et al.* 2014, Šedová *et al.* 2015).

Our aim was to determine the genotypic and allele frequencies of the rs1526538 polymorphism THSD7A gene in the Roma population in Slovakia and to demonstrate its possible association with selected anthropometric indicators of obesity. Subsequently, the results were compared with the majority population of Slovakia.

MATERIALS AND METHODS

The study of genotype frequencies of the polymorphic variant rs1526538 of the THSD7A gene included 407 individuals of the Roma population and 229 individuals of the majority population in eastern Slovakia. All participants signed informed consent and were informed about the course and importance of the research. Samples were extracted from buccal mucosa and genomic DNA isolation was performed according to the protocol of the commercial extraction kit (Promega, Madison, USA). Genotyping was performed according to a standard protocol using a Taqman® SNP genotyping assay (C__2041063_10; Applied Biosystems, Foster City, CA) on an Applied Biosystem Fast 7500 Real-Time PCR machine. Anthropometric measurements of body height (cm), body weight (kg), waist circumference (cm), and hip circumference (cm) were performed by standard methods to all participants, and BMI, WHR and WHtR indexes were calculated. Critical values according to WHO were selected to assess overweight and obesity in the analyzed set – BMI ≥ 25 kg/m^2 and waist circumference >102 cm for men, >88 cm for women. SPSS for Windows version 19.0 (SPSS, Inc., Chicago, IL) was used for statistical data analysis. The Hardy-Weinberg equilibrium was tested using Pearson's chi^2-square test. Association analysis was performed by logistic regression. $P<0.05$ was chosen as the criteriium of statistical significance for all tests.

RESULTS

The basic characteristics of the study groups of the Slovak and the Roma populations are shown in *Table 1*. The mean age was 38.33 (± 13.07) years in the Roma and 40.09 (± 14.66) years in the Slovak population. The mean and median BMI value in both groups was above the overweight. As shown in *Table 2*, significant differences between the Roma and Slovak populations were demonstrated in all obesity indicators: BMI ($p < 0.002$), waist circumference ($p<0.001$), WHR ($p<0.001$) and WHtR ($p<0.001$).

The risk allele A of the rs1526538 polymorphism of the THSD7A gene occurred in the Slovak population in 60.9% and the G allele in 39.1% (*Table 3*). In the Roma group the risk allele frequency was 47.9% and the G allele frequency 52.1%. The genotypic distribution of polymorphism was in Hardy-Weinberg equilibrium. In the Slovak population we detected 24.1% AA homozygotes, 53.7% AG heterozygotes and 12.2% GG homozygotes. The higher frequency of GG homozygotes was in the Roma group (29.0%), AG heterozygotes were 46.2% and AA homozygotes 24.8%. We found statistically significant differences in allele frequency of the polymorphism between the two populations studied ($p<0.001$).

Association analysis (*Table 4*) compared the difference in allele and genotype numbers in obese subjects (BMI ≥ 25 kg/m^2, male waist circumference ≥102 cm and female waist circumference ≥88 cm) and subjects with normal weight. No allele association was found in the Roma (OR=1.063; 95% CI=0.804–1.407; p=0.666) and majority group (OR=1.416; 95% CI=0.971–2.064; p=0.070), equally, there was no statistical association of polymorphism with the waist circumference in both groups [Roma: (OR=0.974; 95% CI=0.740–1.282; p=0.849) Majority: (OR=0.912; 95% CI=0.618–1.345; p=0.641)].

DISCUSSION

Obesity is a chronic and long-standing disease that causes development of many medical complications such as type 2 diabetes mellitus (Al-Goblan *et al.* 2014), cancer (Gallagher, LeRoith 2015), osteoarthritis (Zheng, Chen 2015), dyslipidemia (Klop *et al.* 2013), psychiatric disorders (Strine *et al.* 2002) or cardiovascular disease (Wilson *et al.* 2002). The basic feature of obesity is the imbalance between energy intake and expenditure, resulting in abnormally increased accumulation of adipose tissue. The value of anthropometric parameters is one of the ways how to diagnose or assess obesity and its complications.

In this study, we determined the value of waist circumference, hip circumference, BMI, WHR and WHtR index in the Roma population in comparison with the majority population. We found that in the Roma population there was significant increase in value of all detected obesity parameters in comparison to the majority population sample.

The average BMI value in the monitored Roma population was 27.63 ± 6.64 kg/m^2, which according to the WHO classification would belong into the category of overweight, in the majority population the average value was 25.76 ± 4.79 kg/m^2. The median of the waist circumference as indicator of abdominal obesity was the higher in the Roma population (94.00

TABLE 1: Characteristic of study population. N, number of individuals; SD, standard deviation; Max, maximum; Min, minimum; WC, waist circumference; HC, hip circumference; BMI, body mass index; WHR, waist to hip ratio; WHTR, waist to height ratio; RP, Roma population.

Study parameters	Slovak population (n=229)				Roma population (n=407)			
	Mean	SD	Max.	Min.	Mean	SD	Max.	Min.
Age (years)	40.09	±14.66	69	19	38.33	±13.07	67	19
Height (cm)	169.98	±9.10	193	150	163.06	±9.11	190	136
Weight (kg)	74.59	±16.25	130	43	73.52	±18.58	130	35
BMI (kg/m^2)	25.73	±4.79	40.03	17.21	27.63	±6.64	51.12	15,18
WC (cm)	89.08	±14.38	141	56	95.47	±16.14	140	60
HC (cm) (n=366 RP)	103.06	±10.69	150	73	104.21	±12.24	150	60
WHR (n=366 RP)	0.86	±0.08	1.13	0.63	0.92	±0.09	1.23	0,69
WHTR	0.52	±0.08	0.79	0.34	0.59	±0.10	0.92	0,37

TABLE 2: Comparison of study parameters between groups. N, number of individuals; WC, waist circumference; HC, hip circumference; BMI, body mass index; WHR, waist to hip ratio; WHTR, waist to height ratio; CI, confidence interval; p value, level of significance; RP, Roma population.

Study parameters	Slovak population (n=229)		Roma population (n=407)		p value
	Median	95% CI	Median	95% CI	
Age (years)	40.00	37.00–42.00	36.00	35.00–39.00	0.221
Height (cm)	169.00	168.00–170.00	163.00	162.00–164.00	<0.001*
Weight (kg)	71.00	70.00–75.00	71.00	70.00–74.00	0.343
BMI (kg/m^2)	25.16	24.17–26.22	26.67	25.71–27.68	<0.002*
WC (cm)	88.00	85.00–90.00	94.00	90.00–95.00	<0.001*
HC (cm) (n=366 RP)	102.00	100.00–104.00	103.00	101.00–104.00	0.308
WHR (n=366 RP)	0.87	0.85–0.88	0.91	0.90–0.92	<0.001*
WHTR	0.52	0.51–0.53	0.58	0.57–0.59	<0.001*

TABLE 3: Allele and genotypes frequencies of rs1526538 of THSD7A. N, number of participants; no, number; AA, homozygous genotype; AG, heterozygous genotype; GG, homozygous genotype; HWE, Hardy-Weinberg equilibrium; c^2, chi-square; df, degree of freedom; p, level of significance.

Genotype/Participants	Slovak (n=229)	Roma (n=407)
	(No, %)	
AA	78 (24.1)	101 (24.8)
AG	123 (53.7)	188 (46.2)
GG	28 (12.2)	118 (29.0)
Allele		
A	279 (60.9)	390 (47.9)
G	179 (39.1)	424 (52.1)
HWE	p=0.071	p=0.132
Comparison of genotype frequency	χ^2 = 24.089 Df = 2 p<0.001*	

cm) compared with Slovak majority group (88.00 cm). The results of our work are consistent with the results of other studies that show that the Roma population differs from the majority by their characteristic features, such as a higher prevalence of obesity (Vozarova De Courten *et al.* 2003, Dolinská *et al.* 2007, Mačeková *et al.* 2010, Petrašová *et al.* 2014, Šedová *et al.* 2015). Thus, an active weight reduction would be desirable in the Roma population to avoid multiple health complications.

Obesity is a complex and multifactorial disease determined not only by environmental factors but also by genetic factors (Albuquerque *et al.* 2015). Therefore, molecular – genetic studies to detect hidden genetic markers predisposing individuals to obesity are currently coming to the forefront of interest. In the first place, the pathological locus is identified, the risk alleles are analyzed, and then their pathogenic effect is determined. Whole genome analyzes have considerably expanded the list of obesity candidate genes. One of the recent findings is the discovery of a THSD7A gene variant. Polymorphism rs1526538 of this gene was associated with a higher body mass index in the Indian population. In subjects with one or two risk alleles A, the BMI was significantly higher than in subjects without the risk allele, suggesting that this could be a new obesity gene (Nizamuddin *et al.* 2015). So far there is a little information about the frequency of this polymorphism. Therefore, the aim of our research was to identify and compare allele and genotype frequencies of the rs1526538 polymorphism of the THSD7A gene in the Roma and majority populations of eastern Slovakia and to investigate its possible association with selected indicators of obesity.

Based on the higher prevalence of obesity in the Roma population, we could expect that the frequency of the risk mutant A allele in our Roma sample would be higher compared to the majority population.

In this case, however, the most important factor that plays a role in the development of obesity, given the cultural and health specificities of the Roma ethnic group, is likely to be environmental in nature. Genetic analysis showed that a higher frequency of the A allele was detected in the majority population (60.9%) compared to the Roma population (47.9%). The

TABLE 4: Distribution of genotypes and A allele frequency (±SD, standard deviation) of rs1526538 in overweight and obese individuals (cases) and non-obese individuals (controls) and allele frequency difference with OR (odds ratio) (95%CI (confidence interval)). N, number of individuals; no, number; SD, standard deviation; BMI, body mass index; WC, waist circumstance; AA, homozygous genotype; AG, heterozygous genotype; GG, homozygous genotype; OR, odds ratio; CI, confidence interval; p, level of significance.

Genotype	Slovak population				Roma population			
	Stratified on BMI		Stratified on WC		Stratified on BMI		Stratified on WC	
	Cases N=117	Controls N=112	Cases N=84	Controls N=145	Cases N=240	Controls N=167	Cases N=207	Controls N=200
AA (No, %)	45 (38.5)	33 (29.5)	28 (33.3)	50 (34.5)	62 (25.8)	39 (23.4)	54 (26.1)	47 (23.5)
AG (No, %)	62 (53.0)	61 (54.5)	44 (52.4)	79 (54.5)	109 (45.4)	79 (47.3)	89 (40.0)	99 (49.5)
GG (No, %)	10 (8.5)	18 (16.0)	12 (14.3)	16 (11.0)	69 (28.8)	49 (29.3)	64 (30.9)	54 (27.0)
A allele frequency (±SD)	0.65 (± 0.029)	0.57 (± 0.031)	0.60 (± 0.036)	0.62 (± 0.026)	0.49 (± 0.0024)	0.47 (± 0.028)	0.48 (± 0.026)	0.48 (± 0.025)
OR	1.416		0.912		1.063		0.974	
95% CI	0.971-2.064		0.618–1.345		0.804–1.407		0.740–1.282	
p	0.070		0.641		0.666		0.849	

differences in allele distribution were statistically significant ($p<0.001$). Significant differences between the observed populations ($p<0.001$) were also found in the genotype frequency (majority: AA – 34.1%, AG – 53.7%, GG – 12.2%), (Roma: AA – 24.8%, AG – 46.2%, GG – 29.0%). The baseline data on the frequencies of polymorphisms is provided by the international HapMap database, which was created within the human genome project. The highest frequency of the A allele of the rs1526538 polymorphism (63%) was in the European population (CEU). The lowest A allele frequency (16%) was in people of African descent (YRI). In the Japanese population (JPT) it was 47% and in the Chinese population (CHB) 32% (NCBI 2020).

The results of our study provide initial information on the frequency of this polymorphism in the majority population as well as in the Roma population in eastern Slovakia and confirm that the polymorphic variant of rs1526538 of the THSD7A gene exhibits considerable ethnic variability in frequency. The frequency of the A risk allele found by us in the majority population is the similar as in the Hap Map database for the European population. In a replication association study that was in response to the original finding that it is an obesity-related variant, in the Indian population, the A allele frequency of the rs1526538 polymorphism of the THSD7A gene was 49% (Ahmad *et al*. 2016). Thus, the frequency of this polymorphism in the Roma of Eastern Slovakia is the comparable as that found in the Indian population. Since the origin of the Roma is precisely from the Indian subcontinent, we can assume that there is no change in the allele frequency of this polymorphism in the Roma probably since their migration. The phenotypic parameters of obesity were selected for subsequent allele association analysis. We investigated the relationship of this polymorphism with waist circumference and BMI. Nizamuddin *et al*. (2015) found that in the Indian population the A allele carriers in the homozygous state have significantly higher average BMI than individuals with a single A allele or individuals homozygous for the G allele. Although individuals in the majority population in Slovakia with genotype AA had higher median BMI (25.71 kg/m^2) and waist circumference (89.50 cm) versus individuals with AG (25.16 kg/m^2 resp. 88.00 cm) or GG (23.28 kg/m^2, resp. 87.00 cm), no significant differences were found. Also in the group of subjects with BMI ≥ 25 kg/m^2 there were more carriers of the AA genotype and in the group of subjects with BMI ≥ 25 kg/m^2 there were more carriers of the GG genotype, but again, significant differences were not found in either case – in the comparison of median values between genotypes or in numbers of individual genotypes in a set of cases and controls. Similarly, no significant differences were found in the Roma sample.

Our sample had great age variability (average for Roma: 38.33 ± 13.07 years; min.: 19 years; max.: 67 years), (average for majority: 40.09 ± 14.66 years; min.:

19 years; max.: 69 years). Nizamuddin *et al.* (2015) confirmed the association in a sample of 206 healthy individuals ranging from 20 to 30 years. A subsequent replication study by Ahmad *et al.* (2016) did not confirm association with any anthropometric parameter of obesity, and the population sample of both groups also showed differences (n = 1761: 21 to 63 years and n = 830: 21 to 51 years). With the age increases, the enviromental factors are more prominent in the development of obesity, and thus confirmation of association may be complicated in the samples with significant age variability.

The necessity for further association analysis is likely to be an extension of the group by a larger number of individuals with lower age variability, or association analysis performed in a group of individuals with a more pronounced clinical phenotype, for example with morbid obesity.

At present, it is neither possible to reject nor to confirm the hypothesis of the effect of polymorphic variant rs1526538 on the development of obesity. It is necessary to conduct a similar study in other populations. If this polymorphism does not affect the obese phenotype, we cannot exclude the linkage disequilibrium of this polymorphism with the causal genetic variant.

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