UDC 575
https://doi.org/10.2298/GENSR1801325S
Original scientific paper

INVESTİGATİON OF ASSOCİATİON BETWEEN THE HUMAN NEUROPEPTİDE Y GENE LEUCİNE7PROLİNE POLYMORPHİSM AND OBESİTY İN A POPULATİON FROM TURKEY

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Sözen M. A., Y. Çetinkaya, G. Geçgel, N. Özcan, Ersin Ülger (2018): *Investigation of association between the human neuropeptide y gene leucine7proline polymorphism and obesity in a population from Turkey*.- Genetika, Vol 50, No.1, 325-335.

Obesity, an important public health issue, is a risk factor for many diseases and has been associated with many genes. The aim of this study was to investigate if there is an association between an NPY gene polymorphism, Leu7Pro, and obesity and/or obesity-related phenotypes. A total of 84 obese cases (45 female and 39 male) and a total of 77 non-obese control subjects (38 female and 39 male) were included in this case-control study. Body weight and height measurements were used for calculation of Body Mass Index (BMI) and the ones in the ranges of 18 to 25 kg/m^2 were considered normal and the ones 30 kg/m^2 and over were considered obese from the subjects.

Minor allele frequency for Leu7Pro polymorphism was 3.5% and it was found to be associated to increased obesity in the population studied. No significant differences were observed between genotype distrubutions and allele frequencies for both obese and non-obese subjects. However, mean BMI values were found to be higher (38,88 ± 2,96) in obese cases having Pro7 allele than non carriers of this allele (35,37 ± 5,16), (*p=0.044*). This is the first study in a Turkey population which supports the role of Leu7Pro polymorphism in obesity. Further studies with larger sample sizes may

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confirm these findings and can contribute and shed light on the genetic factors playing a part in Turkey population.

Key words: association analysis, neuropeptide Y gene, *NPY* polymorphism, obesity

INTRODUCTION

Obesity, an important public health issue with its increasing prevalance, is a risk factor for many disorders including cardiovascular diseases, and Type 2 Diabetes (FRIEDMAN, 2000). Besides some rare monogenic forms, obesity is usually inherited in the polygenic, multifactorial mode of inheritance and characterized by a positive energy balance, and increase in the ratio of body fat. It is generally measured by Body Mass Index (BMI).

Many candidate genes have been studied so far in different populations and some of them have been shown to be associated with obesity or obesity related phenotypes (RANKINEN *et al.*, 2006). One of them is Neuropeptide Y (*NPY)* gene, which is localized on chromosome 7 (7p15.1) (ROCHE *et al.*, 1997). and spans 14,678 kb region (NCBI Reference Sequence: NG_016148.1).

NPY is a neurotransmitter that regulates many physiological functions in the body. In animals, Neuropeptide Y (NPY) has been shown to increase food intake and appetite, while it desreases food consumption and thereby leading to positive energy balance and eventually to obesity (PESONEN *et al.*, 2006). According to a model resulting from the studies carried out in model organisms, when there is an increase in body fat, both insulin and leptin levels increases, whereas NPY synthesis and activity is inhibited and food intake decreases. In contrast, when leptin or leptin levels decrease and ghrelin levels increase, NPY synthesis and release increase and this resluts in more food intake (AHIMA *et al.*, 2006; SHINTANI *et al.*, 2001; WOODS *et al.*, 1998).

Association between NPY gene variants and obesity and certain obesity-related phenotypes have been studied in many populations and different findings were obtained. Two studies carried out by Roche *et al.* in French morbidly obese cases and Bhaskar et al. in Indian population have found no evidence supporting the association (ROCHE *et al.*, 1997; BHASKAR *et al.*, 2010). In another study carried out in a population from Brasil have observed that NPY Leu7Pro variant was accounted for the decrease in BMI in premenapousal women (MATTEVİ *et al.*, 2002). VAN ROSSUM et al. have investigated the contribution of *NPY* Leu7Pro variant to body fatness on the basis of obesity prevalance and BMI in a population from Nederland, and found no association in women, however, reported an association between *NPY* 7Pro allele and increased BMI, body weight and leptin levels in men (van ROSSUM *et al.*, 2006). In a study carried out in two Swedish populations an association between Leu7Pro variant and elevated BMI has been found (DING *et al.*, 2005); whereas the same variant was observed to be associated with lower waist-to-hip ratios and higher leptin levels in women (MUTSCHLER *et al.*, 2013). In addition, four *NPY* gene variants including Leu7Pro have been studied and Leu7Pro variant with another polymorphism were found to be associated with elevated risks of obesity from young adulthood to middle age (YEUNG *et al.*, 2011). However, in some populations no such variant were observed at all (MAKINO *et al.*, 2001; DING *et al.*, 2006).

In this study, it was aimed to investigate if there is an association between Leu7Pro polymorphism (rs16147) and obesity and obesity related phenotypes both in men and women in a population from Turkey.

MATERIALS AND METHODS

Subjects

A total of 84 people (45 women and 39 men) whose BMI values are 30 and over (obese group) and 77 (38 women and 39 men) people whose BMI values are between 18,5 to 25 were included in this study. Body weight and height measurements were used for calculation of Body Mass Index (BMI) and the ones in the ranges of 18 to 25 kg/m^2 were considered normal and the ones 30 kg/m^2 and over were considered obese from the subjects. The subjects with Type 2 Diabetes, Cushing syndrome, hypo or hypertiroidism and pregnant women were excluded from the study. The study protocol was approved by the Local Ethics Committee at Afyon Kocatepe University Medical Faculty and all subjects gave their informed consent.

Antropometric Measurements

All antropometric measurements were carried out in the Exercise Physiology lab in the Faculty of Medicine at Afyon Kocatepe University. BMI was calculated as body weight divided by the square of the height (kg/m^2). Body composition parameters were determined by bioelectrical impedance analysis (BIA) system (Bodystat 1500, Bodystat Ltd., Douglas, Isle of Man, UK). The basic premise of BIA procedure is that the volume of fat-free tissue in the body will be proportional to the electrical conductivity of the body (ACSM, 2007). The impedance was measured between the right wrist and right ankle using a tetrapolar electrode system. The subjects lay supine with the arms separated from the body, and with legs not touching each other. Signal electrodes were positioned in the middle of the dorsal surface of the hand and feet proximal to the metacarpophalangeal and metatarsophalangeal joints. Detecting electrodes were more proximally positioned at the ankle and the wrist. An excitation current of 500 μA at 50 kHz was applied to the distal electrodes, and the voltage was detected by the proximal electrodes. The data were analyzed using the manufacturer's software, and body fat percentage, total body fat, lean body mass, body water percentage, total body water and dry lean weight were determined for each subject. Circumference measurements were taken with a 7-mm-wide tape measure while subjects were standing in a straight but relaxed position. The tape measure was held parallel to the ground and completely surrounded the part of the body but did not compress the subcutaneous fat tissue (ACSM, 2007). Duplicate measurements were taken at each site and retests were made if duplicate measurements were not within 7 mm. The sites which reflect central obesity (waist and hip) were used for circumference measurements. The waist/hip ratio was also calculated.

Polymerase Chain Reaction and Genotyping

For PCR amplification genomic DNA's were used as a template extracted using NucleoSpin (Macherey-Nagel) genomic DNA isolation kit. The primers for PCR were as follows: Forward: 5'- CCTGGGTTCTCTCTGCGGGACTG - 3' and Reverse: 5'- CCCATTTTGTGTAGAGTGTGCCCTGT – 3' (BHASKAR *et al.*, 2009). The PCR products amplified then digested with using BSiEI restriction enzyme at 60°C to genotype Leu7Pro (T1128C or rs16139) variant and electrophoresed in %2 agorose gel followed by visualization under UV light. The ones having only one band of 516 bp were genotyped as TT (Leucine) and the ones that have both 516bp and 348, 168'lik bands were genotyped as TC (Leucine/Proline). Afterwards genotype ditributions and allele frequencies were calculated for both obese and non-obese groups.

Statistical Analysis

And obtained results were analyzed statistically using χ^2 test for genotype distributions and Mann–Whitney U test for avarage BMI values to see if there is any significance between cases and controls. All antropometric measurements were carried out in the Physiology lab in the Faculty of Medicine at Afyon Kocatepe University.

RESULTS

Preliminary analyses showed that the Leu7Pro polymorphism was in Hardy-Weinberg equilibrium, in patients and in the controls. Afterwards in order to conduct an association analysis between Leu7Pro polymorphism in *NPY* gene and obesity and obesity related phenotypes, PCR-RFLP analysis and genotyping was carried out for a total of 84 obese and 77 non-obese controls included in this study. *NPY* gene variant was found to be polymorphic in the population studied and its minor alleele frequency was about (3,5%) which is similar to some European countries (DING *et al.,* 2003). And no Pro/Pro homozygotes were observed in this study.

We then compared genotype and allele frequencies for the Leu7Pro polymorphism of *NPY* gene in obese cases versus non-obese controls. For Leu7Pro (1128T>C) polymorphism (Table 1) there was no significant difference in 1128T>C (Leu7Pro) genotype distribution between obese cases and non-obese controls (p=0.73). Likewise, the frequency of 1128C allele was not increased among obese cases as compared with controls (p=0.18). However, when some obesity related phenotypes were compared between two groups (individiulas carrying leucine allele (TT) versus individuals carrying proline allele (TC), there was no significant difference in most antropometric measurements in non-obese group and altogether (Table 2). Interestingly, mean BMI values were found to be significantly higher in cases with TC genotypes who carry Leucine allele (38.88 ± 2.96) than the ones with TT genotypes who carry proline allele (35.37 ± 5.16) in obese group (p=0.044) showing an association between obesity (elevated BMI) and *NPY* Leu7Pro polymorphism, in particular, with 7Pro allele. Also, there was a significant difference in both body fat (kg) and body fat percentage being lower in *NPY* 7Pro allele carriers respectively (p=0.047 and p=0.024).

Table 1. NPY 1128 C>T (L7P) Genotype Distribution and Allele Frequencies in Turkish Obese Cases versus Population-Matched Controls

		Genotype					Allele*		
	n	TT (Leu/Leu)	TC (Leu/Pro)	CC (Pro/Pro)	χ^2	*P*-value	A	C	*P*-value
Controls	84	80 (0.952)	4 (0.048)	0 (0.00)			164 (0.976)	4 (0.024)	
Obese Cases	77	71 (0.922)	6 (0.078)	0 (0.00)	0.63	0.729	148 (0.961)	6 (0.039)	0.178

*Allele data are presented as counts (allele frequency). Genotype *P*-values were calculated by χ^2 (2 df) and allele frequency P-values were calculated by Fisher's exact test, 1-tailed.

Table 2. Demographic and phenotypic data (anthrpopometric measurements) of control and case (obese) groups according to genotype

Variable	Non-Obese Control group			Obese group		
	Genotypes			Genotype		
	Leu/Leu (n=7)	Leu/Pro (n=6)	*P* value**	Leu/Leu (n=80)	Leu/Pro (n=4)	*P* value
Age	28.68±9.944	23.17±3.869	0.170	40.80±9.99	43.50±7.94	0.496
Height	165.63±8.651	167.67±9.136	0.562	163.54±9.287	160.00±8.832	0.421
Weight	62.37±10.582	62.67±11.147	0.902	94.550±14.588	100.00±16.228	0.577
BMI	22.684 ± 2.5502	22,183 ± 2.3413	0.562	35.370 ± 5.167	38.875 ± 2.960	0.044
Waist (cm)	74.076±7.833	73,466±7,818	0.977	97.229±10.452	101.05±8.313	0.693
Hip (cm)	93.336±5.343	91.750±3.267	0.366	111.810±10.386	118.125±3.497	0.052
WHR	0.794±0.0794	0.802±0.0676	0.768	0.873±0.094	0.852±0.045	0.769
Body fat (%)	23.742±7.425	17.566±4.210	0.047	38.957±9.723	44.000±9.556	0.332
Body fat (kg)	14.603±4.678	10.700±1.436	0.024	37.347±12.298	43.200±6.275	0.137
Lean body mass	47.848±10.443	52.133±11.473	0.318	57.540±12.045	56.800±17.142	0.976
Total mass	62.451±10.360	62.833±11.304	0.894	94.523±14.617	100.00±16.227	0.577
Dry lean mass	13.829±4.084	15.617±4.721	0.227	16.003±5.197	13.950±5.285	0.483
Water (%)	54.465±5.497	58.100±3.328	0.085	44.208±5.844	42.375±6.690	0.619
Water (kg)	34.807±9.263	36.517±7.093	0.459	41.694±7.596	42.850±11.952	0.784

*Phenotypic data are presented as mean ± standard deviation.

** P values were calculated using Mann-Whitney U test and P values of <0,05 between compared groups were regarded as significant statistically.

DISCUSSION

Association of *NPY* gene Leu7Pro polymorphism with obesity has been studied in many populations and conflicting results were obtained. Several studies found no association with obesity results (ROCHE *et al.*, 1997; BHASKAR *et al.*, 2010; DING *et al.*, 2003; KARVONEN *et al.*, 1998), while several others found association but with different aspects of obesity. In a study Pro7 allele has been associated with lower BMI values as well as lower WHRs in women respectively (MATTEVİ *et al.*,2002; MUTSCHLER *et al.*, 2013), while in another two studies it was associated with increased BMI values (van ROSSUM *et al.*, 2006; DING *et al.*, 2005) and increased risk of obesity (YEUNG *et al.*, 2011). In addition, van ROSSUM *et al.* reported that Leu7Pro polymorphism was associated with early development of obesity in young adults (van ROSSUM *et al.*, 2006). Moreover, Ding et al. and Yeung et al. also reported that this polymorphism was associated with elevated BMI as well as increased risk for obesity (DING *et al.*, 2005; YEUNG *et al*., 2011). Altogether, these studies suggest that this polymorphism has a role in obesity in some populations. In this study, in terms of genotype distributions and allele frequencies for *NPY* gene Leu7Pro polymorphism no significant difference was found between obese cases and non-obese controls. However, in obese cases, mean BMI values was found to be higher in individuals carrying Pro7 allele than non-carrier of the same allele. Therefore, our results in agreement with the findings that showed an increase in BMI and a role in the predisposition to obesity (van

ROSSUM *et al.*, 2006; DING *et al.*, 2005; YEUNG *et al.*, 2011). These results suggest that Pro7 allele (or another linked marker) might be a risk factor in the development of obesity in the population studied. On the other hand, the significant decrease in both body fat percentage (%) and body fat (kg) being lower in *NPY* 7Pro allele carriers in non-obese group might be relevant to its role with low free fatty acid (FFA) levels in the fasting state and during hyperinsulinemia independently of insulin's antilipolytic effect as well as during exercise as suggested by previous reports (PIHLAJAMAKI *et al.*, 2003; KALLIO *et al.*, 2003). However the physiological significance the of this finding remains to be determined.

Additionally, this study is also the first one in a population from Turkey suggesting an association between obesity and this polymorphism among many predisposing gene variants, though there are others reported such as C.-2548 G>A polymorphism in *Leptin* gene (SAHIN *et al.*, 2013). However, the main limitation of our study is its relatively small sample size. Therefore, while it is limited in its interpretability due to its quite small sample size, it may still serve as a first exploratory step that needs to be followed by further in-depth studies in larger samples from Turkey and other populations to confirm these findings.

In conclusion, the Pro7 allele of the NPY gene is associated with higher BMI in obese subjects and lower body fatness in non-obese subjects. Therefore, this study presents evidence which supports the role of *NPY* gene Leu7Pro polymorphism in obesity. However, it needs to be confirmed further in larger populations to reveal its exact role in obesty.

ACKNOWLEDGEMENTS

This study was supported by Afyon Kocatepe University Scientific Research Projects to M.A.S. (11.TIP.09). We would like to thank Prof. İsmet Doğan for his help on statistical analysis.

Received, February 24th, 2017
Accepted November 18th, 2017

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ISPITIVANJE POVEZANOSTI POLIMORFIZMA HUMANOG NEUROPEPTIDNOG Y GENA LEUCİNE7PROLİNE I GOJAZNOSTI U POPULACIJI IZ TURSKE

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Izvod

Gojaznost, važno pitanje javnog zdravlja, je rizičan faktor za mnog ebolesti i povezan je sa mnogim genima. Cilj istraživanja je ispitivanje da li postoji povezanost između NPY gen polimorfizma, Leu7Pro, i gojaznosti i/ili gojaznost-povezanih fenotipova. Ukupno 84 slučajeva gojaznosti (45 žena i 39 muškaraca) i ukupno 77 ne-gojaznih kao kontrola (38 žena i 39 muškaraca) su uključeni u istraživanje. Težina tela i visina su korišćene za izračunavanje Indeksa mase (Body Mass Index, BMI) i oni u opsegu od 18 do 25 kg/m^2 su razmatrani kao normalni a oni preko 30 kg/m^2 i više kao gojazni.

Minor frekfencija alela za Leu7Pro je 3.5% i povezana je sa gojaznošću u ispitivanoj populaciji. Neznačajna razlika je dobijena između distribucije genotipova i frekfencije alela za obe grupe. Prosečna BMI vrednost je bila veća (38,88 ± 2,96) kod gojaznih koji imaju Pro7 alele nego kod onih koji nemaju (35,37 ± 5,16), (*p=0.044*). Ovo su prva ispitivanjau populaicji iz Turske koja su podržala ulogu Leu7Pro polimorfizma u gojaznosti. Dalja istraživanja na većem uzorku možda potvrde ovo mogu da doprinesu rasvetljavanju genetičkih faktora koji imaju ulogu u populacijama iz Turske.

Primljeno 24.II.2017.
Odobreno 15. I. 2018.