

Trp64Arg POLYMORPHISM OF THE β_3 -ADRENERGIC RECEPTOR AND ITS ASSOCIATION WITH OBESITY IN THE YOUNG POPULATION OF NORTH MACEDONIA

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The pandemic surge of obesity is becoming a concern for civilization. Genetic factors play an important role in obesity. The polymorphism of the *beta3-adrenergic receptor gene (ADRB3)*, whose protein is located mainly in adipose tissue, has been associated with body weight disorders and obesity. The relationship between the *ADRB3* polymorphism and BMI has been controversial. Our research examines the impact of the *Trp64Arg* polymorphism in the *ADRB3* gene on anthropometric and biochemical parameters. Analyzing data from 110 young North Macedonians, we employed PCR-RFLP genotyping alongside biochemical and anthropometric assessments. Obesity was measured using the body mass index (BMI), and weight groups were determined according to World Health Organization (WHO) guidelines. Significant associations emerged between *ADRB3* genotypes and crucial biochemical parameters—glucose, triglyceride, and cholesterol levels. P-values of 0.013, 0.011, and 0.025, respectively, indicate a potential correlation between specific genotypes and variations in these metabolic markers. This pioneering study sheds light on *ADRB3*'s role in metabolic health, offering potential for personalized interventions and emphasizing the need for further research in the North Macedonian population.

Keywords: obesity, *ADRB3* gene, polymorphism, anthropometry, biochemistry

INTRODUCTION

Obesity has emerged as a global health challenge, with a major negative impact on social, economic, and, most importantly, physical and mental health. An examination of World Health Organization (WHO) data for North Macedonia underscores an alarming trend in Body

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Mass Index (BMI) among the youth, revealing an increasing proportion falling within overweight and obese categories (WORLD OBESITY FEDERATION, 2022; WHO/EUROPE, 2021).

Obesity is acknowledged as a multifactorial condition, influenced by various factors, including genetics, nutrition, environmental factors, and behavior (ZIMERE *et al.*, 2020). Over 500 genes and chromosomal regions have been identified in association with human obesity phenotypes, among which the *Adrenergic Beta-3 Receptor (ADRB3)* gene, featuring the *Trp64Arg* polymorphism, has garnered attention for its potential role in weight disorders (TANIKA *et al.*, 2008; EKTA *et al.*, 2022; SARAH *et al.*, 2013; HÉLÈNE and DAVID, 2011; JESSICA and MERLIN, 2022).

The *Adrenergic Beta-3 Receptor (ADRB3)* is an essential part of the adrenergic signaling system, impacting processes such as catabolic activity, thermogenesis, and lipid mobilization. As part of the G-protein coupled receptor family, *ADRB3* is predominantly expressed in adipose tissue, playing a central role in governing energy expenditure and lipid metabolism. [9]

Genetic variations within the *ADRB3* gene, referred to as *ADRB3* polymorphisms, have garnered attention for their potential role in influencing an individual's susceptibility to metabolic and weight disorders (RAJAN KUMAR *et al.*, 2017; AKIKI *et al.*, 2012). These polymorphisms in *ADRB3*, as well as in *ADRB1* and *ADRB2*, have been associated with obesity (AKIKI *et al.*, 2012). Specifically, *ADRB3* is instrumental in hydrolyzing triacylglycerides into fatty acids, impacting fat accumulation in the body.

One well-known polymorphism is the *Trp64Arg* variant, where the amino acid tryptophan is replaced by arginine at position 64 of the beta-3 adrenergic receptor. The association between this polymorphism and obesity has undergone extensive investigation, yielding both supportive and conflicting findings. Some studies have suggested a potential link between this polymorphism and obesity, insulin resistance, and other metabolic disturbances, while others have found no significant associations. There are controversial results even in the same population (SARAFINA *et al.*, 2011; SHAUHEI *et al.*, 2012; NAOYUKI, 2011; YUMI *et al.*, 2003).

KATALIN *et al.* (2015) investigated the association of common polymorphisms of genes with energy expenditure and obesity in 528 Hungarian school-aged children. One of them was *Trp64Arg* of β 3-adrenoreceptor (*ADRB3*) polymorphism. They concluded that carriers of the *ADRB3 Arg 64* allele had a higher body mass index. More studies are done on the Japanese population, and the results have been controversial (SHAUHEI *et al.*, 2012; YUMI *et al.*, 2003; YOHEI *et al.*, 2021). YUMI *et al.*, (2003) and AYA *et al.* (2010) have not found a significant relationship between *ADRB3* polymorphism and BMI. In contrast, SHOUHEI *et al.* (2012) have reported that annual weight has been significantly associated with the *Arg* allele of *ADRB3*. Similar results were shown in a recent study by KEI *et al.* (2019). They concluded that carriers of the heterozygous genotype *Trp64Arg* show more attraction to high-calorie foods with high levels of fat and sugar compared to homozygous carriers.

In our study, we conducted an analysis to explore the potential association between the *Trp64Arg* polymorphism in the *ADRB3* gene and obesity-related phenotypic variation within the young North Macedonian population.

MATERIAL AND METHODS

The study included 110 subjects (45% men and 55% women), young North Macedonians aged from 18 to 36 years inclusive. Informed written consent was obtained from all study participants before their involvement. *ADRB3* polymorphism, biochemical status, and anthropometric data were analyzed for the participants. Blood collection took place at 'Idadija Medical Center' and 'Cento Medical Center' in Skopje.

Biochemical parameters, including glucose, total cholesterol, and triglycerides, were determined on the ChemWell2902 analyzer device in the PHC-PI-Biochemical laboratory using kits with standards for validated methods. Anthropometric data, including height, weight, waist and hip circumference, and triceps skinfold thickness measured using a skinfold caliper, were recorded. Weight groups were determined based on the calculated body mass index (BMI) using threshold values for adults from WHO, categorizing participants into normal weight, overweight, and obese.

Both blood and a buccal swab were collected from each subject. Genomic DNA was extracted from each subject using a commercial kit (Promega kit) according to the protocol. Part of the DNA samples was isolated with an automated method. Genotyping (TT, TC, or CC) of the *ADRB3* Trp64Arg (T/C) polymorphism was performed by the PCR-RFLP method (polymerase chain reaction - restriction fragment length polymorphism), Figure 1. PCR amplifications for *ADRB3* genes were performed under conditions published in our previous paper (ZIMERE *et al.*, 2019)."

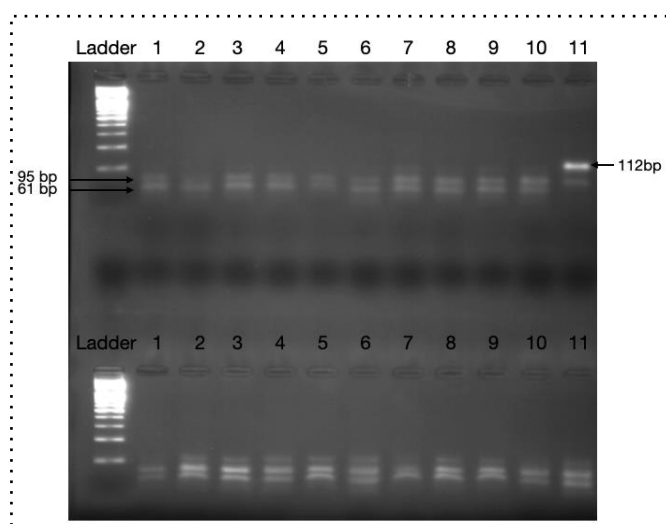


Figure 1. RFLP patterns of PCR product of *ADRB3* digested with *MvaI* (BstNI)

Lane 1 - lane 8: PCR products of the human Trp64/Trp64 (64, 34) . Lane 9: fragments of human Arg64/Trp64 (95, 64, 34) Lane 11: PCR products of a human; not digested (112 bp)

Statistical analyzes were conducted with the R programming language and a p-value of 0.05 was considered statistically significant. Specifically, we used Fisher's Exact Test to determine the statistical significance of the relationship between genotype and weight groups. ANOVA technique was used to assess differences among genotype categories for various anthropometric and biochemical parameters.

RESULTS AND DISCUSSION

In the final statistical analysis of the *ADRB3* gene, 101 subjects were included. However, the results of the PCR-RFLP analysis in the remaining 10 subjects were not clearly readable, prompting their exclusion from the study. The frequencies of alleles and genotypes were calculated and categorized according to the three groups, as presented in Table 5. Genotype frequencies were found to be concordant with Hardy-Weinberg expectations.

Table1. Genotype frequencies of the ADRB3 Trp64Arg polymorphism in normal weight, overweight and obese subjects.

ADRB3			
Genotype	Normal	Overweight	Obese
Arg / Arg	7	4	
Trp / Arg	15	11	9
Trp / Trp	33	19	3

Genotyping in 101 subjects revealed the distribution of *ADRB3 Trp64Arg* polymorphism in different weight groups. In the normal weight group, 33 individuals (60%) were homozygous for the wild-type allele (Trp64Trp), 15 (27.3%) were heterozygous for the variant allele (Trp64Arg), and 7 (12.5%) were homozygous for the variant allele (Arg64Arg). Similar distribution patterns were observed in the overweight group, where 19 individuals (55%) were homozygous for the wild-type allele, 11 (32.4%) were heterozygous, and 4 (11.8%) were homozygous for the variant allele. In the obese group, the majority were heterozygous carriers for the variant allele (Trp64Arg), accounting for 9 individuals (70%), while 3 (30%) were homozygous for the wild-type allele (Trp64Trp), and none were homozygous for the variant allele (Arg64Arg).

For Fisher's test, a p-value of 0.05 was obtained, while the Chi-squared test gave a p-value of 0.03. These p-values suggest a significant association between genotypes and body weight groups.

Table 2. Phenotypes of subjects divided according to genotyping of the ADRB3 gene

Phenotypic Variables	Arg / Arg	Trp / Arg	Trp / Trp	p-value
BMI	23.1 ± 0.31	25 ± 0.52	23.58 ± 0.34	0.217
Waist	78.36 ± 1.44	83.97 ± 1.73	79.16 ± 1.15	0.252
W/H ratio	0.8 ± 0.01	0.82 ± 0.01	0.79 ± 0.01	0.538
Tricep skinfold	19 ± 0.53	23.84 ± 0.85	22.33 ± 0.76	0.190
Glucose	4.84 ± 0.07	5.09 ± 0.03	4.77 ± 0.05	0.013
Triglycerides	0.78 ± 0.05	1.11 ± 0.06	0.77 ± 0.05	0.011
Cholesterol	4.82 ± 0.06	4.81 ± 0.11	4.32 ± 0.08	0.025

Additionally, groups were categorized according to their genotypes and correlated with their phenotypic characteristics using ANOVA, as outlined in Table 2.

The carriers of the Arg allele, specifically those with the Trp/Arg genotype, exhibited higher mean values for BMI, glucose, triglyceride, cholesterol, waist circumference, hip circumference, and waist-to-hip ratio compared to individuals with the Arg/Arg and Trp/Trp genotypes. No statistically significant differences were observed in the relationship between anthropometric measurements and ADRB3 genotypes. Statistically significant associations were identified with biochemical parameters, including glucose (p-value: 0.013), triglyceride (p-value: 0.011), and cholesterol levels (p-value: 0.025). These findings indicate statistically significant associations between carriers of the Arg allele, especially in the Trp/Arg genotype, and key metabolic markers.

To our knowledge, this is the first-ever investigation into the association between the *Trp64Arg* polymorphism in the *ADRB3* gene and metabolic disorders in the young North Macedonian population. Motivated by the absence of existing data, our pioneering research provides essential insights into the role of *ADRB3* in metabolic health. Our findings suggest that *ADRB3*'s impact is more pronounced at the metabolic level than in directly affecting anthropometric outcomes.

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Trp64Arg POLIMORFIZAM β_3 -ADRENERGIČKOG RECEPTORA I NJEGOVA ASOCIJACIJA SA GOJAZNOŠĆU KOD MLADOG STANOVNIŠTVA SEVERNE MAKEDONIJE

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Izvod

Nalet pandemije gojaznosti postaje briga civilizacije. Genetski faktori igraju važnu ulogu u gojaznosti. Polimorfizam gena *beta3-adrenergičkog receptora (ADRB3)*, čiji se protein nalazi uglavnom u masnom tkivu, povezan je sa poremećajima telesne težine i gojaznošću. Odnos između *ADRB3* polimorfizma i BMI je kontroverzan. Naše istraživanje ispituje uticaj polimorfizma *Trp64Arg* u genu *ADRB3* na antropometrijske i biohemijske parametre. Analizirajući podatke od 110 mladih iz Severne Makedonije, koristili smo PCR-RFLP genotipizaciju uz biohemijske i antropometrijske procene. Gojaznost je merena korišćenjem indeksa telesne mase (BMI), a grupe težine su određene u skladu sa smernicama Svetske zdravstvene organizacije (SZO). Pojavile su se značajne veze između *ADRB3* genotipova i ključnih biohemijskih parametara—glukoze, triglicerida i nivoa holesterola. P-vrednosti od 0,013, 0,011 i 0,025, respektivno, ukazuju na potencijalnu korelaciju između specifičnih genotipova i varijacija u ovim metaboličkim markerima. Ova pionirska studija baca svetlo na ulogu *ADRB3* u metaboličkom zdravlju, nudeći potencijal za personalizovane intervencije i naglašavajući potrebu za daljim istraživanjem u populaciji Severne Makedonije.

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