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eNOS G894T and ACE I/D gene polymorphisms and their association with anthropometric and biochemical parameters

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Abstract

The primary objective of this study was to analyze the frequency of alleles and genotypes of the G894T polymorphism of the endothelial nitric oxide synthase (eNOS) gene and the insertion-deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene in relation to body mass index (BMI) and biochemical parameters. The study included 64 subjects. Analysis of genotype distribution of the eNOS G894T polymorphism in relation to BMI showed that the GT+TT genotype was present in 11 subjects with BMI ≤ 24.9 and in 25 subjects with BMI ≥ 25. For the ACE I/D polymorphism, the ID+DD genotype was detected in 10 subjects with BMI ≤ 24.9 and in 36 subjects with BMI ≥ 25. No statistically significant differences in allele or genotype frequencies were observed between BMI groups ($p > 0.05$). Regarding biochemical parameters, although the differences lacked statistical significance ($p > 0.05$), GT+TT genotypes (eNOS G894T) were more frequent in subjects with elevated glucose, total cholesterol, and triglyceride levels compared to the GG genotype. Similarly, the analysis revealed no statistically significant association between the ACE I/D polymorphism and the examined biochemical parameters ($p > 0.05$). A major limitation of the present study is the relatively small sample size; therefore, these findings should be considered preliminary. Nevertheless, the specific trends observed in genotype distribution represent an important indicator in investigating the potential role of these polymorphisms in the genetic background of the metabolic profile. Thus, further research on larger and more homogeneous cohorts is necessary to elucidate this potential significance.

Keywords

eNOS, ACE, BMI, biochemical parameters

Introduction

According to the WHO, overweight and obesity are defined as excessive fat accumulation that may present a health risk. In addition to environmental factors such as diet and lifestyle, genetic predisposition also plays an important role in the pathophysiology of obesity. Endothelial nitric oxide synthase (eNOS) and angiotensin-converting enzyme (ACE) are involved in the regulation of vascular tone and blood pressure. Polymorphisms such as eNOS G894T and ACE insertion/deletion (I/D) have been associated with cardiovascular risk factors, including obesity and hypertension (Šljivančanin-Jakovljević, 2019; Birhan et al., 2022). Numerous studies have aimed to identify genetic polymorphisms that may contribute to the development of obesity. Nitric oxide plays an important role in maintaining the functional integrity of the vascular endothelium and in regulating vascular tone and metabolic homeostasis (Gamil et al., 2017). The nitric oxide synthase (NOS) enzyme family consists of three isoforms: neuronal NOS (nNOS; NOS1), inducible NOS (iNOS; NOS2), and endothelial NOS (eNOS; NOS3), each characterized by distinct patterns of expression and physiological functions (Šljivančanin-Jakovljević, 2019). Among these, isoform NOS3 is the predominant isoform in the vascular endothelium and is therefore of particular importance for cardiovascular regulation. It is encoded by the NOS3 gene, which is highly polymorphic and includes single-nucleotide polymorphisms (SNPs), variable number tandem repeats (VNTRs), microsatellites, and insertion/deletion variants (Oliveira-Paula et al., 2017). The NOS3 gene is located on chromosome 7 and spans approximately 21-22 kb, containing 26 exons and 25 introns (Marsden et al., 1993).

Due to its biological relevance, the eNOS G894T (rs1799983) polymorphism has been investigated in relation to obesity and associated metabolic disturbances. Previous studies suggest that this variant may be associated with obesity susceptibility, BMI, and metabolic risk profiles in certain populations (Nasr et al., 2016). Its effect may be more evident in interaction with clinical conditions such as obesity and type 2 diabetes mellitus, rather than as an independent genetic determinant (Bressler et al., 2013).

Moreover, variation in the eNOS gene has been linked to the relationship between serum cholesterol levels and blood pressure, supporting a possible interaction between genetic background, lipid metabolism, and vascular regulation (Pereira et al., 2006). The ACE gene is located on the long arm of chromosome 17, specifically in the 17q23.3 region. The ACE I/D polymorphism is characterized by the insertion or deletion of a 287 bp Alu sequence within intron 16, resulting in three genotypes II homozygous, ID heterozygous, and DD homozygous (Sameer et al., 2010; Sakuma et al., 2004). Due to its biological role in the renin-angiotensin system, this polymorphism has been investigated in relation to hypertension, obesity, and metabolic disturbances. Costa et al. (2021) reported that the ACE I/D polymorphism, particularly the DD genotype, had previously been associated with excess body weight and obesity. Although this association was not pronounced when ACE was analysed individually, it became significant when examined in combination with the ACTN3 polymorphism, suggesting a possible polygenic contribution to the development of obesity. Liu et al. (2024) further reported that the ACE I/D polymorphism was associated with certain lipid profile parameters, particularly the TG/HDL-C ratio, while associations with HDL-C and LDL-

C levels were also observed in women. Furthermore, Heidari-Beni et al. (2025), in a systematic review and meta-analysis, found that the ACE I/D polymorphism was significantly associated with anthropometric parameters, especially body mass index (BMI), with carriers of the DD genotype exhibiting higher mean BMI values compared to carriers of the ID and II genotypes. Collectively, these findings suggest that ACE gene variants may contribute to interindividual differences in body weight and lipid metabolism. While previous studies in the Tuzla Canton (Hadžibeganović et al., 2017; Avdić et al., 2022; Alić et al., 2023) have investigated the association of eNOS and ACE polymorphisms with coronary artery disease and hypertension, this study is a preliminary investigation focusing primarily on their correlation with BMI and biochemical parameters.

Material and methods

Study population and design

This study included 64 participants of both sexes, all older than 18 years. All participants in the study were fully informed about the study protocol, and gave informed consent in accordance with the Declaration of Helsinki. The research was also approved by the Ethics Committee for Scientific Research, University of Tuzla. Sample collection for DNA isolation, anthropometric measurements for BMI assessment, survey data collection, and biochemical analyses were conducted at the Private health institution - Polyclinic - EsoLab, Lukavac, with institutional approval. Biochemical analyses, anthropometric measurements, subject selection, and sample collection for DNA isolation were performed as part of routine daily examinations.

Biochemical analyses

Standard laboratory methods were used to determine glucose, total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels. Determination of all biochemical parameters was performed on the ERBA XL-200 automatic analyzer, according to the principles of good laboratory practice, with daily internal quality control (IQC) and monthly external quality assessment (EQA).

Genomic DNA isolation and PCR-RFLP genotyping

DNA isolation was performed using the commercial kit PureLink Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's protocol (Thermo Fisher Scientific). Molecular genetic analysis of the eNOS G894T polymorphism for all subjects was performed using an optimized method based on PCR-RFLP, using a set of primers 5'-CATGAGGCTCAGCCCCAGAAC-3' (sense) and 5'-AGTCAATCCCTTTGGTGCTCAC-3' (antisense) (Dasar et al., 2012). Amplification was performed on a polymerase chain reaction device (Applied Biosystems by Life Technologies, 2720 Thermal Cycler).

The reaction was carried out in 30 cycles, and after that amplified PCR products were subjected to restriction digestion with MboI restriction enzyme according to the manufacturer's protocol, which was further optimized. Fragments with a length of 206 bp (GG), 206, 119, 87 bp (GT) and 119, 87 bp (TT) were obtained (Dasar et al., 2012; Arif Yalcin et al., 2014). Analysis of restriction fragments was performed by electrophoresis on a 3.5% agarose gel. Molecular genetic analysis of the ACE I/D polymorphism was performed using an optimized

method based on PCR, using a set of primers 5'-CTGGAGACCACTCCCATCCTTTCT-3' (sense) and 5'-GATGTGGCCATCACATTCGTCAGAT-3' (antisense) (Birhan et al., 2022; Tamiru et al., 2018; Rigat et al., 1992). The reaction was performed in 30 cycles; fragments of 490 bp (II), 190 bp (DD) and 490 and 190 bp (ID) were obtained. Analysis of PCR products was performed by electrophoresis on a 2% agarose gel (Birhan et al., 2022; Tamiru et al., 2018; Krishnan et al., 2016; Rigat et al., 1992).

Data processing and statistical analyses

Statistical analysis was performed using MedCalc 12.4.0.0 and SPSS 29.0.0.0 software. Hardy-Weinberg equilibrium (HWE) for genotype distributions was assessed using the chi-square test. Bivariate associations and odds ratios (ORs) with 95% confidence intervals (CIs) were evaluated using chi-square and Fisher's exact tests. Furthermore, a binary logistic regression model was employed to determine the independent effects of the studied polymorphisms on biochemical and anthropometric parameters, yielding adjusted ORs while controlling for potential covariates. A p-value of <0.05 was considered statistically significant.

Results and discussion

The present study evaluated the relationship between allele and genotype frequencies of the eNOS G894T and ACE I/D polymorphisms and selected anthropometric and biochemical parameters in 64 subjects. Demographic analysis revealed that most participants were over 50 years of age (n = 47; 73.44%), while 17 (26.56%) were under 50. Genetic diversity analysis (He) confirmed pronounced variability for both observed loci, with values of 0.418 for eNOS G894T and 0.500 for ACE I/D. The genotype distribution for the ACE I/D polymorphism was in complete agreement with the Hardy -Weinberg equilibrium (HWE) (p = 0.621). In contrast, a statistically significant deviation from HWE was recorded for eNOS G894T (p = 0.029). Given the limited sample size and the notably low observed frequency of TT homozygotes (n = 2), a more robust exact test (Exact HWE test) was additionally applied. The exact test confirmed the presence of a significant deviation (p = 0.038), indicating that the observed discrepancy primarily reflects the specific distribution within the analyzed sample. The results of eNOS G894T genotyping are presented in Figure 1, where the successful electrophoretic separation

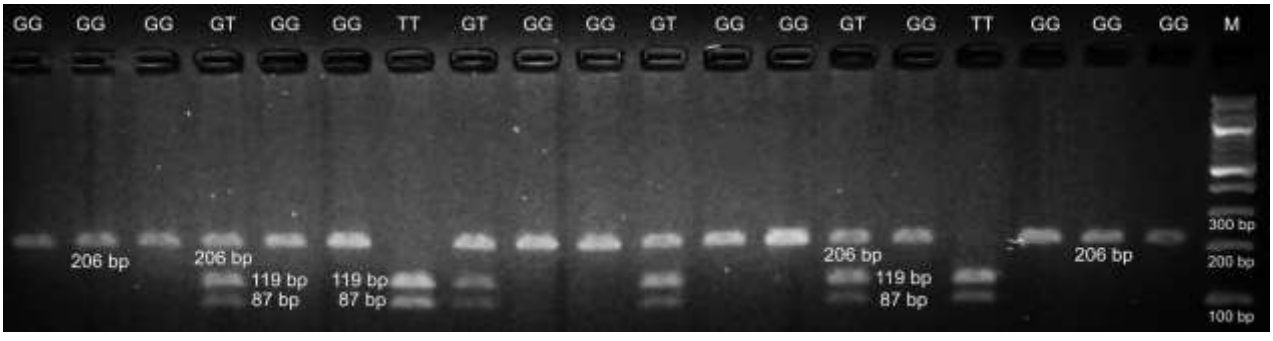

Figure 1. Representative agarose gel electrophoresis of eNOS G894T polymorphism. Lane M: 100 bp DNA ladder; Lanes GG, GT, TT: identified genotypes

of restriction fragments confirms the presence of the three expected genotypes: GG, GT, and TT. The distribution of eNOS G894T alleles across all BMI categories reveals a higher prevalence of the G allele compared to the T allele (Table 1).

The distribution of genotypes and alleles for the eNOS G894T polymorphism in relation to the analyzed biochemical parameters is shown in Table 2. Although the GT genotype was more commonly observed among subjects with elevated glucose, total cholesterol, and triglyceride levels, these differences did not reach statistical significance ($p > 0.05$). Therefore, no significant association was confirmed between the eNOS G894T polymorphism and the analyzed metabolic parameters in the present sample.

However, these findings should be interpreted in the context of existing literature. Teixeira et al. (2016) reported that the eNOS Glu298Asp polymorphism influences body composition in obese elderly women, while a meta-analysis by Luo et al. (2019) suggested that the association between the NOS3 rs1799983 variant and coronary artery disease may be partially mediated by T allele related alterations in lipid metabolism. The lack of significance in our study is likely attributable to its preliminary nature and the relatively small sample size, which limits overall statistical power. Genotyping of the ACE I/D polymorphism was performed by direct visualization of PCR products on agarose gel, where fragments of 490 bp (allele I) and 190 bp (allele D) were identified, corresponding to homozygous (II and DD) and heterozygous (ID) genotypes (Figure 2). Table 3 showed that the combined ID+DD genotypes were more common among subjects with BMI ≥ 25 kg/m². However, the higher frequency of D allele carriers (ID+DD) was not statistically significant ($p > 0.05$), confirming no direct association between the ACE I/D polymorphism and elevated BMI.

The observed frequency of the I allele (51.56%) (Table 3) falls within the reference values documented for European populations, according to data available in meta-analyses and population genetic databases (CDC Genomics and Precision Health Knowledge Base; Wang et al., 2014). Analysis of the ACE I/D polymorphism (Table 4) indicated a high prevalence of D allele carriers (ID and DD genotypes) within both reference and elevated biochemical parameter groups.

Table 1. Genotype and allele frequencies of the eNOS G894T polymorphism in relation to body mass index (BMI) categories

***eNOS* G894T genotype**	**BMI (N%)**						
	18.5-24.9	**25.0-29.9**	**≥ 30.0**	**Total**			
GG	5 (31.25%)	13 (56.52%)	10 (40.00%)	28 (43.75%)			
GT	11 (68.75%)	8 (34.78%)	15 (60.00%)	34 (53.13%)			
TT	0 (0.00%)	2 (8.70%)	0 (0.00%)	2 (3.12%)			
Total	16	23	25	64			
	< 25		**≥ 25**	**Total**	$\chi 2$	p	p*
GG	5 (31.25%)		23 (47.92%)	28 (43.75%)	1.354	0.244	0.383
GT+TT	11 (68.75%)		25 (52.08%)	36 (56.25%)	OR (95% CI)	p	
Total	16		48	64	2.024 (0.610-6.715)	0.239	
Alleles	**< 25**		**≥ 25**	**Total**	OR (95% CI)	p	p*
G	21 (65.63%)		69 (71.87%)	90 (70.31%)	0.747 (0.318-1.756)	0.504	0.510
T	11 (34.37%)		27 (28.13%)	38 (29.69%)			
Total	32		96	128			

BMI-body mass index; OR-odds ratio; CI-confidence interval; p-chi-square test; p*-Fisher's exact test.

Table 2. Genotype and allele frequencies of the eNOS G894T polymorphism in relation to clinical biochemical parameters

eNOS G894T genotype	Biochemical parameters			
	Glucose > 6.1 mmol/L	**Glucose ≤ 6.1 mmol/L**	**Total cholesterol > 5.2 mmol/L**	**Total cholesterol ≤ 5.2 mmol/L**
GG	8 (33.33%)	20 (50.00%)	14 (36.84%)	14 (53.85%)
GT	16 (66.67%)	18 (45.00%)	23 (60.53%)	11 (42.30%)
TT	0 (0.00%)	2 (5.00%)	1 (2.63%)	1 (3.85%)
GG	8 (33.33%)	20 (50.00%)	14 (36.84%)	14 (53.85%)
GT+TT	16 (66.67%)	20 (50.00%)	24 (63.16%)	12 (46.15%)
Total	24	40	38	26
Alleles	**Glucose > 6.1 mmol/L**	**Glucose ≤ 6.1 mmol/L**	**Total cholesterol > 5.2 mmol/L**	**Total cholesterol ≤ 5.2 mmol/L**
G	32 (66.66%)	58 (72.50%)	51 (67.10%)	39 (75.00%)
T	16 (33.33%)	22 (27.50%)	25 (32.90%)	13 (25.00%)
Total	48	80	76	52
	Triglycerides ≥ 1.7 mmol/L	**Triglycerides < 1.7 mmol/L**	**LDL ≥ 3.0 mmol/L**	**LDL < 3.0 mmol/L**
GG	12 (42.86%)	16 (44.44%)	18 (50.00%)	10 (35.71%)
GT	16 (57.14%)	18 (50.00%)	17 (47.22%)	17 (60.71%)
TT	0 (0.00%)	2 (5.56%)	1 (2.78%)	1 (3.58%)
GG	12 (42.86%)	16 (44.44%)	18 (50.00%)	10 (35.71%)
GT+TT	16 (57.14%)	20 (55.56%)	18 (50.00%)	18 (64.29%)
Total	28	36	36	28
Alleles	**Triglycerides ≥ 1.7 mmol/L**	**Triglycerides < 1.7 mmol/L**	**LDL ≥ 3.0 mmol/L**	**LDL < 3.0 mmol/L**
G	40 (71.43%)	50 (69.44%)	53 (73.61%)	37 (66.07%)
T	16 (28.57%)	22 (30.56%)	19 (26.39%)	19 (33.93%)
Total	56	72	72	56

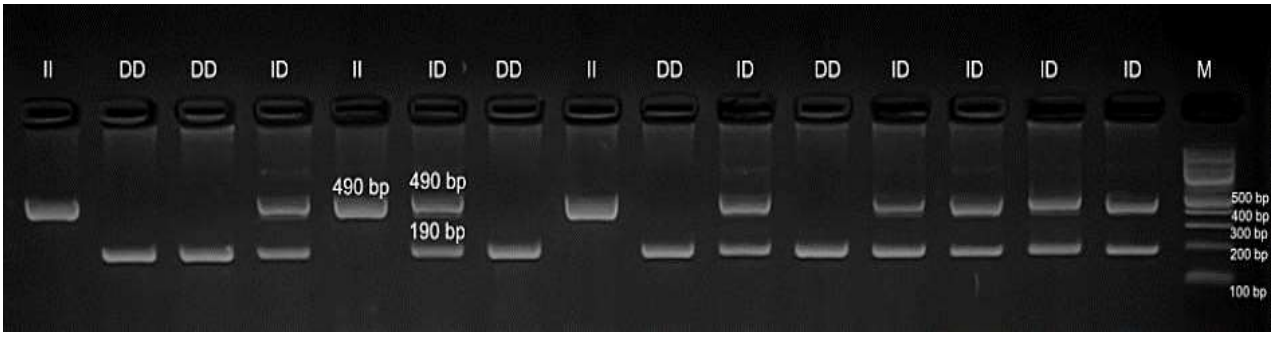

Figure 2. Representative agarose gel electrophoresis showing ACE gene I/D genotyping
Lane M: 100 bp DNA ladder; II, ID, and DD: representative genotypes

However, the observed distribution trends did not reach the threshold of statistical significance, thereby failing to confirm a direct correlation within this cohort.

Age-group analysis indicated a clearer distribution pattern for the ACE I/D polymorphism than for eNOS G894T. A borderline difference in ACE genotype distribution was observed between individuals < 50 and ≥ 50 years ($p = 0.083$; Fisher's $p = 0.075$). The DD genotype and D allele were significantly more frequent in the ≥ 50 group ($p = 0.034$ and $p = 0.029$, respectively). No significant differences were found for eNOS G894T. These findings suggest a possible age-related effect for

ACE I/D, although they should be interpreted cautiously due to the limited sample size. The analysis of combined eNOS G894T and ACE I/D genotypes showed that GT/ID was the most frequent combination, observed in 16 subjects (25.00%).

The findings of the matched pair study conducted by Seyedrezazadeh et al. (2021) indicate that the T allele (p = 0.02), GT genotype (p = 0.03), and the dominant model (TT+GT vs. GG, p = 0.01) are associated with an increased risk of metabolic syndrome (MetS).

Table 3. Genotype and allele frequencies of the ACE I/D polymorphism in relation to body mass index (BMI) categories

***ACE* I/D genotype**	**BMI (N%)**						
	18.5-24.9	**25.0-29.9**	**≥30.0**	**Total**			
II	6 (37.50%)	4 (17.40%)	8 (32.00%)	18 (28.13%)			
ID	5 (31.25%)	11 (47.82%)	14 (56.00%)	30 (46.87%)			
DD	5 (31.25%)	8 (24.78%)	3 (12.00%)	16 (25.00%)			
Total	16	23	25	64			
	< 25	**≥ 25**		**Total**	χ2	p	p*
II	6 (37.50%)	12 (25.00%)		18 (28.13%)	0.928	0.336	0.352
ID+DD	10 (62.50%)	36 (75.00%)		46 (71.87%)	OR (95% CI)		
Total	16	48		64	0.556 (0.167-1.853)	0.344	
Alleles	**< 25**	**≥ 25**		**Total**	OR (95% CI)	p	p*
I	17(53.13%)	49 (51.04%)		66 (51.56%)	1.087 (0.488-2.423)	0.838	1.000
D	15 (46.87%)	47 (48.96%)		62 (48.44%)			
Total	32	96		128			

Table 4. Genotype and allele frequencies of the ACE I/D polymorphism in relation to clinical biochemical parameters

***ACE* I/D genotype**	**Biochemical parameters**			
	Glucose > 6.1 mmol/L	**Glucose ≤ 6.1 mmol/L**	**Total cholesterol > 5.2 mmol/L**	**Total cholesterol ≤ 5.2 mmol/L**
II	7 (29.17%)	11 (27.50%)	13 (34.21%)	5 (19.23%)
ID	13 (54.17%)	17 (42.50%)	15 (39.47%)	15 (57.70%)
DD	4 (16.67%)	12 (30.00%)	10 (26.32%)	6 (23.07%)
II	7 (29.17%)	11 (27.50%)	13 (34.21%)	5 (19.23%)
ID+DD	17 (70.84%)	29 (72.50%)	25 (65.79%)	21 (80.77%)
Total	24	40	38	26
Alleles	**Glucose > 6.1 mmol/L**	**Glucose ≤ 6.1 mmol/L**	**Total cholesterol > 5.2 mmol/L**	**Total cholesterol ≤ 5.2 mmol/L**
I	27 (56.25%)	39 (48.75%)	41 (53.95%)	25 (48.08%)
D	21 (43.75%)	41 (51.25%)	35 (46.05%)	27 (51.92%)
Total	48	80	76	52
	Triglycerides ≥ 1.7 mmol/L	**Triglycerides < 1.7 mmol/L**	**LDL ≥ 3.0 mmol/L**	**LDL < 3.0 mmol/L**
II	10 (35.71%)	8 (22.22%)	11 (30.56%)	7 (25.00%)
ID	13 (46.43%)	17 (47.22%)	16 (44.44%)	14 (50.00%)
DD	5 (17.86%)	11 (30.56%)	9 (25.00%)	7 (25.00%)
II	10 (35.71%)	8 (22.22%)	11 (30.56%)	7 (25.00%)
ID+DD	18 (64.29%)	28 (77.78%)	25 (69.44%)	21 (75.00%)
Total	28	36	36	28
Alleles	**Triglycerides ≥ 1.7 mmol/L**	**Triglycerides < 1.7 mmol/L**	**LDL ≥ 3.0 mmol/L**	**LDL < 3.0 mmol/L**
I	33 (58.93%)	33 (45.84%)	38 (52.77%)	28 (50.00%)
D	23 (41.07%)	39 (54.16%)	34 (47.23%)	28 (50.00%)
Total	56	72	72	56

This is further supported by a pronounced association of the TT genotype in the male subpopulation ($p = 0.01$) and a significant contribution of NOS3 and BMI variables in the CART analysis. In contrast, our study revealed no statistically significant difference in the distribution of both eNOS and ACE genotypes across the BMI groups of the participants ($p > 0.05$). Nevertheless, it should be emphasized that the limited sample size likely reduced the statistical power of the test, preventing the observed trends from reaching statistical significance. Similarly, Pan et al. (2016) and Almeida et al. (2017) found no statistically significant association between the ACE I/D polymorphism and obesity, despite observed differences in genotype distribution and metabolic parameters among the studied groups. Motawi et al. (2016) found no significant association between the ACE I/D polymorphism and obesity in an Egyptian cohort, a comprehensive meta-analysis by Heidari-Beni et al. (2025) reported that the DD genotype is significantly associated with higher mean BMI values.

The application of adjusted binary logistic regression analysis in our study demonstrated that the eNOS G894T polymorphism does not exhibit a statistically significant association with the categories BMI $\geq$ 25 kg/m²or BMI $\geq$ 30 kg/m². Similarly, the ACE I/D dominant and recessive models were not significantly associated with BMI $\geq$30 kg/m², while the ACE dominant model also showed no significant association with BMI $\geq$ 30 kg/m². The only statistically significant finding was observed for the ACE I/D recessive model in relation to BMI $\geq$ 30 kg/m², where the DD genotype was associated with lower odds of obesity compared to the II+ID genotypes (adjusted OR = 0.22; 95% CI: 0.05 -0.96; $p = 0.044$) after adjusting for eNOS G894T, age group, sex, and smoking status. Although a trend toward lower odds was observed in the ACE dominant model for BMI $\geq$ 30 kg/m² this result did not reach statistical significance ($p = 0.149$). Given the limited sample size and the wide confidence interval, this significant finding should be interpreted with caution and warrants confirmation in larger studies. Among the analyzed comparisons, the lowest p-values for the eNOS G894T polymorphism were observed in relation to total cholesterol and glucose, whereas for the ACE I/D polymorphism, the allele distribution according to triglyceride levels was closest to the threshold of significance. Additional stratification by smoking status revealed only minor descriptive variations, which should also be interpreted with caution due to the small number of subjects in individual subgroups. A higher prevalence of the GT+TT (eNOS) genotypes among participants with elevated glucose and lipid levels may indicate a potential role of these polymorphisms in metabolic and vascular disorders, which aligns with previous studies linking eNOS variants to endothelial dysfunction and target organ damage (Śladowska-Kozłowska et al., 2015).

While trends of increased frequencies of risk genotypes (GT+TT) for the eNOS polymorphism were observed among participants with elevated biochemical parameters, the distribution of ACE variants remained uniform across all studied groups. These findings suggest that the biological impact of these genetic markers should be viewed through the prism of their potential interaction and cumulative contribution, rather than solely as independent risk factors within the studied cohort. These observations may have a biological basis in the complex synergism between the renin-angiotensin system (RAS) and nitric oxide (NO) signaling. Specifically, increased RAS activity,

mediated by ACE and angiotensin II, can promote enhanced production of reactive oxygen species (ROS) via NADPH oxidase activation. This process directly results in reduced NO bioavailability, thereby accelerating the development of endothelial dysfunction and metabolic disorders (Griendling et al., 2000). Given the central role of nitric oxide (NO) in regulating vascular homeostasis and insulin signaling, the disruption of NO-mediated pathways can contribute to decreased insulin sensitivity and metabolic dysfunction, as described in previous review studies (Muniyappa & Sowers, 2013; Tabit et al., 2010). Metabolic parameters, such as BMI and lipid profile, represent complex, polygenic traits influenced by numerous genetic and environmental factors, where the small effect sizes of individual polymorphisms may hinder the detection of significant associations in studies with a limited sample size (Cardon & Bell, 2001).

The application of adjusted binary logistic regression analysis in our study revealed no statistically significant associations between the eNOS G894T polymorphism and elevated levels of glucose, total cholesterol, triglycerides, or LDL. Similarly, the ACE I/D polymorphism in both the dominant and recessive models was not significantly associated with elevated biochemical parameters. A borderline association was observed between the ACE dominant model and elevated triglycerides ($p = 0.061$); however, this finding did not reach statistical significance. The remaining analyzed associations did not yield significant results. Overall, none of the evaluated genetic models served as an independent, statistically significant predictor of elevated biochemical parameters after adjusting for age, sex, smoking status, and the other polymorphism included in the model.

The population genetic relevance of this study is reflected in the specific demographic background of the Tuzla Canton population. Western Balkan populations are characterized by a complex genetic structure shaped by historical migrations, with documented distinctions compared to other European populations (Kovačević et al., 2014). Given the general deficit of data regarding cardiometabolic markers in Bosnia and Herzegovina, our results on the distribution of eNOS and ACE polymorphisms contribute to completing the European genetic map. The significance of this genetic profile gains further importance within the context of specific environmental factors of the Tuzla region. As a long standing industrialized area with high levels of air pollution, this population is chronically exposed to oxidative stress, which has been proven to induce endothelial dysfunction (Brook et al., 2010). Such environmental pressure can directly modulate the functional expression of indigenous eNOS and ACE genotypes, suggesting that the monitored parameters (such as BMI and glucose) are the result of complex gene-environment interactions specific to the studied population (Franks et al., 2013).

PCR-RFLP is a cost-effective and reliable method for genotyping known SNP variants. However, it is limited by the requirement of a specific restriction site and its focus solely on the targeted locus (Kwok & Chen, 2003). Modern alternatives, such as SNP microarrays and next-generation sequencing (NGS), offer high-throughput analysis and maximum resolution, enabling the detection of rare and novel mutations genome-wide (LaFramboise, 2009; Shendure & Ji, 2008). Nevertheless, for well-characterized polymorphisms such as ACE I/D and eNOS G894T, conventional PCR approaches remain a valid and

standard choice (Yang et al., 2021; Siregar et al., 2020), particularly when accuracy is validated via Sanger sequencing of a sub-sample (Kwok & Chen, 2003). To mitigate methodological limitations, future research on the Tuzla Canton population should implement advanced sequencing technologies for a broader and more detailed genetic analysis.

Conclusions

Although no significant associations were observed between eNOS G894T and ACE I/D polymorphisms and the analyzed anthropometric and biochemical parameters, genotype trends may suggest a modest contribution of nitric oxide and renin-angiotensin pathways to metabolic variability. These findings provide preliminary insight into metabolic and cardiovascular risk factors in the Tuzla Canton population, warranting further investigation in larger studies.

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Authors' contributions

E. Kurtović:
Formal analysis; Investigation; Methodology; Resources.

A. Avdić:
Conceptualization; Formal analysis; Methodology; Supervision; Roles/Writing – original draft; Writing – review & editing.

M. Šehić:
Data curation; Formal analysis; Methodology; Visualization; Writing – review & editing.

V. Bešlić:
Data curation; Formal analysis; Visualization.

S. Širanović:
Data curation; Roles/Writing – original draft; Writing – review & editing.

A. Hercegovac:
Roles/Writing – original draft; Writing – review & editing.

D. Husejnagić:
Methodology.

E. Burgić:
Resources.

Dž. Osmanović:
Roles/Writing – original draft.

N. Mandžić:
Formal analysis.

Conflict of interest

Authors declare no conflict of interest.

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