Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Research Article Open access

Association between insertion/deletion polymorphism of the *ACE* gene with risk of hypertension

Jovan Kulić[1], Milena Dubravac Tanasković[1], Milan Kulić[1], Bojan Joksimović[1], Ajla Smajlović[2], Radmila Balaban Đurević[1], Nikolina Elez-Burnjaković[1]

[1] *Faculty of Medicine Foča, University of East Sarajevo, Foča, Bosnia and Herzegovina*
[2] *Genetic Association in Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina*

DOI: 10.31383/ga.vol7iss2ga02

***Correspondence**
E-mail: kulicjovan@yahoo.com

Received
November, 2023
Accepted
November, 2023
Published
December, 2023

Copyright: ©2023 Genetics & Applications, The Official Publication of the Institute for Genetic Engineering and Biotechnology, University of Sarajevo

Keywords

ACE gene polymorphisms, Hypertension, Genetic markers, INDEL polymorphisms

Abstract

This study conducted an initial investigation into the association between *ACE* gene insertion/deletion (I/D) polymorphisms (rs1799752) and hypertension in the Republic of Srpska, Bosnia and Herzegovina. The study featured two distinct groups, each with 100 subjects, systematically categorized based on hypertension status and gender. DNA was extracted, PCR-amplified, and analyzed by gel electrophoresis. Results revealed a higher prevalence of the DD genotype and the D allele in the hypertensive group, although statistical significance was not observed. The II genotype occurred in 18% of the hypertension group and 21% in the control group. A significant difference was found in allele I frequencies between the two groups (p=0.004), with no gender-related variations in *ACE* alleles. The limited sample size may have constrained the ability to detect statistically significant differences. The odds ratio for the (DD + ID) genotype compared to II was 1.2110 (95% CI: 0.6006 to 2.4418; p=0.5927), indicating no statistical significance. Furthermore, no significant associations were identified between *ACE* genotypes and alleles and gender. In summary, this preliminary study suggests a potential trend towards a higher prevalence of the *ACE* gene D allele and DD genotype in hypertensive individuals. However, due to the small sample size, these associations did not achieve statistical significance in this population. Larger-scale investigations are needed for more definitive insights into the relationship between *ACE* gene polymorphisms and hypertension.

Introduction

Hypertension stands as a significant modifiable risk element associated with renal, cardiovascular, and cerebrovascular diseases. Furthermore, it ranks as a prominent underlying contributor to global mortality and morbidity. The intricate nature of blood pressure's pathophysiology has been gradually unfolding, encompassing the interplay of genetic and environmental factors in conjunction with an array of physiological pathways and mechanisms, all culminating in the observed phenotype. Although epidemiological investigations have enhanced our comprehension of the impact of environmental factors, particularly diet and exercise, on blood pressure, the precise contribution of genetics remains a complex challenge, particularly when disentangling it from the shared environment often present within families and communities (Patel et al., 2017).

The *ACE* gene codes for the enzyme Angiotensin I-Converting Enzyme, which plays a crucial role in the regulation of blood pressure and electrolyte balance. Its primary function involves catalyzing the conversion of Angiotensin I into the physiologically active peptide Angiotensin II. Angiotensin II is a potent vasoconstrictor and a peptide that stimulates aldosterone, a hormone responsible for controlling blood pressure and fluid-electrolyte balance. Notably, this Angiotensin-Converting Enzyme (ACE) also inactivates the vasodilatory protein bradykinin (National Center for Biotechnology Information, n.d.).

The most extensively studied polymorphism in the ACE gene that affects ACE serum levels is rs1799752 (synonyms: rs4340, rs13447447, rs4646994). This polymorphism involves the insertion/deletion of an Alu repetitive element in an intron. Alleles containing the insertion are denoted as I alleles, whereas D alleles lack the repetitive element. Three genotypes exist: D/D, I/D, and I/I, with the highest *ACE* levels found in the D/D genotype, intermediate levels in I/D, and the lowest levels in I/I genotype based on research done by Tomita et al. (1997).

The aims of this study were to identify the D/D, I/I and I/D genotypes of the *ACE* gene in the population of Republic of Srpska with hypertension, and to investigate whether there are differences in the frequencies of genotypes and alleles for the ACE gene polymorphisms between individuals with hypertension and control subjects.

Material and methods

Participants

The study involved 200 participants, divided into two groups, each consisting of 100 individuals. The first group comprised individuals with hypertension, while the second group, the control group, consisted of individuals without hypertension. Venous blood was collected from the participants using vacutainers with EDTA anticoagulant, transported on ice, and frozen at -86°C until extraction.

Samples were collected between December 2021 and January 2022 in the territory of Republic of Srpska, spanning from Bijeljina to Trebinje. All participants provided their signature in the consent form to participate in the research, which was approved by the Ethics Committee of the Faculty of Medicine, University of East Sarajevo. They were interviewed, and blood was drawn for research purposes, while respecting bioethical principles and ensuring the privacy of the participants involved in the study.

DNA extraction and genotyping

Genomic DNA was isolated from full venous blood using a commercial kit (DNeasy, Blood & Tissue kit (250), Qiagen), following the manufacturer's instructions. The isolated DNA was frozen and stored at -86°C in a freezer at the Center for Biomedical Research at the Faculty of

Medicine in Foča.

The DNA concentration was determined using a commercial kit (Qubit dsDNA BR Assay Kit, Thermo Fisher Scientific). and the Qubit 4 fluorimeter (Thermo Fisher Scientific).

The sequence of primers used to amplify the ACE gene were obtained according to Yoshida et al. (1995). The sequence of forward primer used was: F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and the reverse primer R: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'.

The reaction was conducted using a thermocycler (Eppendorf Mastercycler Personal) in a final volume of 20μL according to Zmorynski et al (2019). PCR conditions were as followed: one cycle of denaturation (94°C for 5 minutes), followed by 35 amplification cycles consisting of denaturation (94°C for 30 seconds), primer annealing (58°C for 45 seconds) and extension (72°C for 30 seconds) and one cycle of final extension (2 minutes at 72°C). PCR products size was checked using 2% agarose gel electrophoresis and visualized under UV light (Vilber FUSION Solo X). After gel visualization, three possible genotypes were observed, I/D genotype with 2 fragments (190 bp and 490 bp), I/I genotype with 1 fragment of 490 bp and D/D genotype with 1 fragment of 190 bp.

Statistical analysis of data

Descriptive and analytical statistical methods were employed in this study. Descriptive statistics methods were used for categorical variables, with relative numbers utilized. Odd's ratio (OR) and 95% interval confidence (CI) were applied to assess risk factors with online MedCalc calculator (MedCalc's Odds ratio calculator). Analytical statistical methods were employed to assess the significance of differences, specifically non-parametric $\chi 2$ tests. The statistical data analysis was conducted using the SPSS software package, version 26.0 ("Statistical Package for Social Sciences," SPSS 26.0 Inc, USA). P-value of ≤ 0.05 was considered significant.

Results and Discussion

Out of the total number of participants, 101 (50.5%) were male, and 99 (49.5%) were female. The study included 100 participants who reported having hypertension during the survey and 100 participants without hypertension.Allele and genotype frequencies were determined in the study. In the total analyzed population, the distribution of *ACE* gene genotypes was as follows: 77 (38.5%) were homozygotes with the D/D genotype, 84 (42%) were heterozygotes with the I/D genotype, and 39 (29.5%) were homozygotes with the I/I genotype. Allele D frequency was higher (59.5%), while allele I frequency was lower (40.5%) in total analyzed population.

Among the groups of participants divided by gender, there was no significant difference in hypertension frequency. Similarly, no statistically significant difference in *ACE* gene genotypes was observed between the groups of participants divided by gender. In the hypertension group, there were 45 individuals with the D/D genotype, 37 with I/D genotype, and 18 with I/I genotype. In the control group, there were 32 D/D genotypes, 47 I/D genotypes, and 21 I/I genotypes (Table 1). *ACE* gene genotypes were compared between groups of participants divided by the presence of hypertension, no significant difference in the frequency of D/D, I/D, and I/I genotypes of the *ACE* gene was observed. Although the D/D *ACE* genotype was more common in participants with hypertension (45%) compared to control participants (32%), the difference was at the threshold of statistical significance ($p=0.059$) (Table 1). No significant association was obtained between ACE I/D genotypes and hypertension risk (Table 2).

The frequency of allele D in the hypertension group was 127, accounting for 63.5%, while allele I was 73, accounting for 36.5%. In the control group, the frequency of allele D was 111,

accounting for 55.5%, and allele I was 89, accounting for 44.5%.

A statistically significant difference in the presence of I allele ($\chi 2$=8.408; p=0.004) was observed between the groups of participants divided based on the presence of hypertension, while there was no significant difference in the frequency of D allele. Control group participants (70%) had a significantly higher occurrence of I allele compared to participants with hypertension (56%) (Table 1). A meta-analysis conducted in 2021, by Liu et al. (2021), involving 57 studies with over 30,000 participants aimed at investigating the relationship between *ACE* gene polymorphisms and hypertension, concluded that the *ACE* gene D allele is associated with hypertension, both in the homozygote model, dominant model, and recessive model. It was also found that the Asian population with the *ACE* gene D allele exhibited a stronger association with hypertension in all these models. Furthermore, it was concluded that the *ACE* gene D allele is closely associated with increased susceptibility to hypertension in subgroups of the Caucasian population and mixed-race populations. In these subgroups, males were in the allele model, homozygote model, and recessive model, while most females were in the allele model.

The association of these gene polymorphisms with hypertension seems to vary between races, nationalities, geographical regions, etc. Supporting this, one study in Japan, conducted by Sugiyama et al. (1999) found no correlation between I/D polymorphism of the *ACE* gene, neither between men and women nor between controls and individuals with hypertension. In contrast, another studies in India demonstrated an association between the D/D polymorphism and essential hypertension (Das et al., 2008; Krishnan et al., 2016).

So far, a few studies in Bosnia and Herzegovina have involved the *ACE* gene, but none of them analyzed the association between hypertension and *ACE* polymorphisms (Tomić et al., 2020; Emir et al., 2017; Ćenanović et al., 2017). This makes our study unique, as findings on the distribution of *ACE* gene alleles and genotypes are of paramount importance.

The research we conducted revealed an increased frequency of I allele of the *ACE* gene in the control group compared to the group with hypertension. Furthermore, it was observed that the D/D genotype was more common in the group of participants with hypertension compared to the control group, with the difference being at the threshold of statistical significance. Therefore, a larger sample analysis may be needed to provide a more detailed insight into the frequency of genotypes.

Conclusion

The most valuable result of our study is distribution of *ACE* gene polymorphism in analyzed population. In this study, differences in the genotypic and allelic frequencies of ACE gene polymorphism between controls and patients were obtained, but insignificant, except statistically significant presence of I allele in the control group, among groups of participants with hypertension and controls.

A limitation of our study is relatively small sample size. Future studies, with a larger number of participants, and additional markers can help better understand the significance of *ACE* I/D polymorphism in pathology of hypertension.

Conflict of Interest

None to declare.

Acknowledgments

This research was conducted as part of the project "Connection between individual genetic susceptibility and severity of clinical presentation of SARS-CoV-2 infection," ID number UIS/MFF: V.2.22-4, led by Assist. Prof. Nikolina Elez-Burnjaković, and funded by the Faculty of

Medicine Foča, University of East Sarajevo. The practical part of the work was carried out in the Laboratory for Molecular Genetics, Center for Biomedical Sciences, Faculty of Medicine Foča.

References

ACE angiotensin I converting enzyme [Homo sapiens (human)] - gene - NCB. National Center for Biotechnology Information. U.S. National Library of Medicine. Available at: https://www.ncbi.nlm.nih.gov/gene/1636#summary [Accessed February 2, 2023].

Cenanovic M, Dogan S, Marjanovic D (2017) Diversity of ACE and ACTN3 Polymorphisms in Bosnian-Herzegovinian Populations. In 10th ISABS Conference on Forensic and Anthropologic Genetics and Mayo Clinic Lectures in Individualized Medicine.

Das M, Pal S, Ghosh A (2008) Angiotensin converting enzyme gene polymorphism (insertion/deletion) and hypertension in adult Asian Indians: a population-based study from Calcutta, India. Human Biology, 80(3):303-12.

Emir M, Karolina S.-Ż, Amina V, Nevena M, Anna P.-M, Ewa C, Damir M, Grażyna A (2017) Lack of association between I/D ACE and -675 ID 4G / 5G PAI-1 polymorphisms and predicting the risk of pregnancy loss (PROPALO) in Bosnian women. IFMBE Proceedings 440–444.

Krishnan R, Sekar D, Karunanithy S, Subramanium S (2016) Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with essential hypertension in the South Indian population. Genes & Diseases, 3(2):159-163.

Liu M, Yi J, Tang W (2021) Association between angiotensin-converting enzyme gene polymorphism and essential hypertension: A systematic review and meta-analysis. Journal of Renin-Angiotensin-Aldosterone System 22(1), 1470320321995074.

Patel RS, Masi S, Taddei S (2017) Understanding the role of genetics in hypertension. European Heart Journal 38(29):2309-2312.

Sugiyama T, Morita H, Kato N, Kurihara H, Yamori Y, Yazaki Y (1999) Lack of sex-specific effects on the association between angiotensin-converting enzyme gene polymorphism and hypertension in Japanese. Hypertension Research 22(1):55-9.

Tomic N, Osmanbegovic A, Mujala A, Prtvar D, Hadzic M, Jasmin R, Lojo-Kadric N, Pojskic N, Pojskic L (2020) ACE DD genotype is associated with high visceral sensitivity index score in a healthy student population in Bosnia and Herzegovina [Preprint]. doi:10.21203/rs.3.rs-23103/v1.

Tomita H, Ina Y, Sugiura Y, Sato S, Kawaguchi H, Morishita M, Yamamoto M, Ueda R (1997) Polymorphism in the angiotensin-converting enzyme (ACE) gene and sarcoidosis. American Journal of Respiratory and Critical Care Medicine 156(1):255-9.

Yoshida H, Mitarai T, Kawamura T, Kitajima T, Miyazaki Y, Nagasawa R, Kawaguchi Y, Kubo H, Ichikawa I, Sakai O (1995) Role of the deletion polymorphism of the angiotensin-converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. Journal of Clinical Investigation 96(5): 2162-9.

Zmorzynski S, Szudy-Szczyrek A, Popek-Marciniec S, Korszen-Pilecka I, Wojcierowska-Litwin M, Luterek M, Chocholska S, Styk W, Swiderska-Kołacz G, Januszewska J, Mielnik M, Hus M, Filip AA (2019) ACE Insertion/Deletion Polymorphism (rs4646994) Is Associated With the Increased Risk of Multiple Myeloma. Frontiers in Oncology 9:44.

Table 1. Distribution of genotypes and alleles among groups of participants divided by genders and according to the presence of hypertension

Groups of participants	Gender of participants				Cases and controls			
	Males	Females	x2	p	HTA	Controls	x2	p
Genotypes								
D/D	36 (35.64%)	41 (41.4%)	0.703	0.402	45 (45,0%)	32 (32.0%)	3.569	0.059
I/D	48 (47.52%)	36 (34.4%)	2.557	0.110	37 (37.0%)	47 (47.0%)	2.035	0.152
I/I	17 (16.8%)	22 (22.2%)	0.644	0.422	18 (18.0%)	21 (21.0%)	0.136	0.713
Alleles								
D	120 (59.4%)	118 (59.6%)	2.193	0.139	127 (81.5%)	111 (79.0%)	0.394	0.530
I	82 (40.6%)	80 (40.4%)	1.194	0.163	73 (56.0%)	89 (70.0%)	8.480	0.004

Table 2. The associations between *ACE* I/D genotypes and hypertension risk.

Genotypes	Participants with HTA	Controls	OR (95% CI)	p-value
II and DD	63	53		
ID	37	47	1,5 (0.8583 to 2.6562)	0.15
Total	100	100		
II and ID	55	68		
DD	45	32	0,57 (0.3233 to 1.0231)	0.05
Total	100	100		
ID and DD	82	79		
II	18	21	1,21 (0.6006 to 2.4418)	0,59
Total	100	100		

OR: Odds ratio, 95% Cl: 95 % confidence interval.