

# ACE DD Genotype Is Associated with High Visceral Sensitivity Index Score in Healthy Student Population in Bosnia and Herzegovina

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## Research

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# Abstract

## Background

Visceral Sensitivity Index (VSI) questionnaire measures gastrointestinal specific anxiety a mediator of the relationship between general psychological distress measures and gastrointestinal symptom severity. Studies have shown that angiotensin converting enzyme (ACE) may be required for sympathoadrenal activation during stress. The aim of our study was to explore the relationship of *ACE* gene polymorphisms with the scores for self-reported visceral hypersensitivity in the sample of student population exposed to psychological distress.

## Methods

A blood sample was taken from ninety students during exam period. DNA was isolated and genotyping of *ACE* polymorphism (rs1799752) was performed using PCR method. The PCR products were analysed on a 2% agarose gel. All respondents completed the VSI questionnaire and based on the scores were stratified into two comparison groups. Allele and genotype association was tested using Fisher's Exact Test in WINPEPI.

## Results

Respondents with total score of up to 65 were classified in the first group and with values over 65 in the second group. Increased frequencies of D allele and DD genotype were observed in the subgroup of students with higher VSI score.

## Conclusions

Obtained results revealed statistically significant association of allele D and DD genotype with increased VSI score. Our results indicate that further genetic and genome studies of regulation of brain-gut axis and visceral hypersensitivity could be helpful in clinical interpretation of their impact on functional gastrointestinal disorders (FGID) symptoms and on development of some other acute and chronic stress related conditions in youth population.

## Background

Functional gastrointestinal disorders (FGID) are common class of disorders in gastroenterology. There is growing number of patients suffering from the FGIDs worldwide but is little known about actual causes and prevalence especially in developing countries. <sup>(1)</sup> The symptoms of FGIDs are heterogeneous, tend to come and go over time, and can overlap with many symptoms of other gastrointestinal disorders. Many patients state the worsening of the disease in stressful conditions, difficulties in work or marriage. There

has been observed comorbidity with some other disorders of gastrointestinal tract but also with some non-gastrointestinal disorders. <sup>(2)</sup>

Visceral hypersensitivity is one of the major characteristics of FGIDs. It usually manifests as pain associated with bowel disturbances. People with visceral hypersensitivity have reduced pain and discomfort thresholds.<sup>(3)</sup> There are several possible underlying mechanisms that have been proposed: subtle inflammation, psychosocial factors and altered sensorimotor function of the gut.<sup>(3)</sup> Visceral sensitivity index has been developed for measuring gastrointestinal symptom-specific anxiety (GSA) and accompanying hypersensitivity.<sup>(4)</sup> It was observed that gastrointestinal symptom-specific anxiety could be a significant predictor of gastrointestinal complaints and an important factor for quality of life in patients with IBS as well for gastrointestinal symptom severity. <sup>(5-6)</sup> Gastrointestinal-specific anxiety is also considered to be a mediator of the relationship between general psychological distress measures and gastrointestinal (GI) symptom severity. <sup>(7)</sup>

Clinical classification of FGIDs is mainly symptom based and in recent years much attention is paid on genetic research for identification of causative pathways and molecular basis.<sup>(8-10)</sup> The population of students is the healthiest segment of one society, but in recent years there has been a constant increase in their morbidity and mortality as well as of chronic diseases and disorders, deformities, growth and development disorders, injuries and mental health problems.<sup>(11)</sup> So called "academic stress" can leave serious and long-term consequences including functional gastrointestinal disorders that are increasingly associated with populations exposed to stress.<sup>(12)</sup>

Stress is very often associated with various functional gastrointestinal disorders, so among the candidate genes associated with these disorders there are also genes whose products are involved in various physiological stress pathways and regulation of brain-gut axis. <sup>(12)</sup>

*ACE* gene encodes for angiotensin converting enzyme which is of vital importance for blood pressure regulation. ACE enzyme is involved in the conversion of angiotensin I to angiotensin II, physiologically active peptide and a potent vasoconstrictor that controls blood pressure and fluid-electrolyte balance.<sup>(13)</sup> Many other physiologic processes are affected by ACE and its peptide substrates and products.<sup>(13)</sup> Effects of angiotensin II are noticed on the vasculature, the heart, the kidney, the nervous system, metabolism, cell proliferation, and a lot of other processes.<sup>(13)</sup> Several studies have shown that ACE may also be involved in hypothalamic-pituitary-adrenal axis (HPA axis) regulation and catecholamine production by generation ATII and therefore required for sympatoadrenal activation during stress.<sup>(14)</sup>

The aim of this study was to explore the association between self-reported scores of Visceral Sensitivity Index questionnaire and the *ACE* genotype for rs1799752 (insertion/deletion in intron 16), among students exposed to psychological distress during the exam period.

## Methods

Ninety students (73,2% female, 26,8% male) aged 18 to 25, self-reported as healthy with absence of chronic or familial diseases, from the University of Sarajevo participated in this study during the summer exam period. The ethical aspects of research project were considered by the Ethics Committee of the Institute of Genetic Engineering and Biotechnology (INGEB) University of Sarajevo and was in accordance with the Helsinki Declaration of 1975 as reflected in a priori approval by the institution's human research committee. Every participant signed written informed consent for voluntary participation in the study and prior to sampling for individual genotyping or phenotyping. A blood sample in the amount of 1–5 µl was taken in the 2 ml test tube from each participant with sterile finger pricking method.

In order to qualify for visceral sensitivity phenotype status, all respondents independently completed the Visceral Sensitivity Index (VSI) questionnaire.<sup>(4)</sup> It measures gastrointestinal specific anxiety (GSA) using 15 questionnaire items. Responses range from 1 (*strongly agree*) to 6 (*strongly disagree*). Previously established methods suggest that VSI is “reverse scored” with item scores ranging from 0 to 5 and total scores ranging from 0 (*no GI-specific anxiety*) to 75 (*severe GI-specific anxiety*).<sup>(4)</sup> Based on the VSI indexing scores, all participants were stratified into two comparison groups. Respondents with total score of up to 65 were classified in the first group (control) and with values over 65 in the second (test) group (Table 1).

Total cell DNA used in genetic analysis was isolated from blood using the custom protocol of Miller et al.<sup>(15)</sup> Genotyping of *ACE* polymorphism (rs1799752) was performed using PCR method with the forward primer 5' CTG GAG ACC ACT CCC ATC CTT TCT 3' and reverse primer 5' GAT GTG GCC ATC ACA TTC GTC AGA 3' (BioTeZ Berlin-Buch GmbH, Germany).<sup>(16)</sup> The following cycling conditions were set: 35 cycles of 94 °C for 5 min, 94 °C for 30 s, 57 °C for 45 s, 72 °C for 45 s and 72 °C for 5 min. The PCR products were analyzed on a 2% agarose gel.

Allele and genotype association was tested using Fisher's Exact Test in WINPEPI.<sup>(17)</sup>

## Results

After stratification, based on VSI scores, 39 out of 90 respondents belonged to the category with low score or complete absence of visceral hypersensitivity symptoms (which is defined as a control group). The rest of 51 of the respondents belonged to category with more expressed visceral hypersensitivity symptoms (which is defined as a test group). Allele and genotype distribution in the studied population are shown in summary in Table 1 with increased frequencies of D allele and DD genotype in the subgroup of students with higher (>65) VSI score. Fisher Exact test for D allele revealed statistically significant association with increased VSI score (one-tailed Fisher's P=0.017). Notably, higher VSI score is positively associated with *ACE* DD genotype (Fisher's P= 0.0386).

**Table 1.** Allele and genotype frequencies for *ACE* gene polymorphism (rs1799752) in total and stratified population of healthy students from Bosnia and Herzegovina

	Total N(%)	Test group N(%)	Control group N (%)	P values
<b>N (%)</b>	90 (100)	51 (100)	39 (100)	
<b>Genotype II</b>	15 (16,67)	6 (11,76)	9 (23,07)	P=0.0386
<b>Genotype ID</b>	36 (40)	17 (33,33)	19 (48,72)	
<b>Genotype DD</b>	39 (43,33)	28 (54,91)	11 (28,21)	
<b>Allele I</b>	66 (37)	29 (28)	37 (47)	P=0.007
<b>Allele D</b>	114 (63)	73 (72)	41 (53)	

## Discussion

Different physiological, psychosocial factors, genetic and environmental factors are included in pathophysiology of functional gastrointestinal disorders. Nowadays, it's confirmed that psychological factors, such as stress, anxiety or depression, are truly important in development of various functional GI disorders such as irritable bowel syndrome (IBS), functional dyspepsia, functional abdominal pain, abdominal migraine, cyclical vomiting syndrome, etc. They may be considered rather as confounding risk factors, than causative. They can, together with social conditions and early life events, make gastrointestinal symptoms worse and define the severity of illness as well as the clinical outcome. <sup>(18)</sup>

The brain-gut axis is one of the most commonly studied features of FGIDs. It refers to neural and hormonal signaling between the central nervous system and the gastrointestinal tract. <sup>(19)</sup> Dysregulation of the brain-gut axis is associated with the pathophysiology of GI tract primarily through a visceral hypersensitivity. <sup>(18)</sup> Visceral hypersensitivity is a multifactorial process that plays a principal role in the etiology of GI symptoms and can occur within the peripheral or central nervous systems. <sup>(20)</sup> It is believed that visceral hypersensitivity in patients causes increased pain and stress symptoms in response to normal bowel activity.

Considering the role of *ACE* gene in the hypothalamic–pituitary–adrenal axis (HPA) regulation and sympatico - adrenal activation during stress, and the role of visceral hypersensitivity in FGIDs, it is not surprising that results of this study show association of higher Visceral Sensitivity score and *ACE* gene polymorphism. <sup>(14, 18–20)</sup> The predictive role of *ACE* polymorphism in persons subjected to additional risk factors (psychological distress, unbalanced diet, etc) should be further investigated as a possible measure in prevention of FGIDs in adolescent population.

## Conclusion

Stress disturbs the balance of the autonomous nervous system (ANS) leading to its excessive excitation and inhibition. Somatization symptoms are an introduction to the development of a clinical manifested disease in the «locus minoris resistentiae» area. It is not possible to prevent stress, but its intensity can be reduced through improving personal organizational skills, communication, physical activity, as well as through familiarization with personal «locus minoris resistentiae» and acting on the same. In that respect, further genetic and genome studies of regulation of brain-gut axis and visceral hypersensitivity could be helpful in clinical interpretation of their impact on FGID symptoms and on development of some other acute and chronic stress related conditions in youth population.

## List Of Abbreviations

VSI - Visceral Sensitivity Index

ACE - angiotensin converting enzyme

FGID - functional gastrointestinal disorders

GI - gastrointestinal

GSA - gastrointestinal symptom-specific anxiety

HPA - hypothalamic–pituitary–adrenal

IBS - irritable bowel syndrome

ANS - autonomous nervous system

## Declarations

### Ethics approval and consent to participate

The ethical aspects of research project were considered by the Ethics Committee of the Institute of Genetic Engineering and Biotechnology (INGEB) University of Sarajevo and was in accordance with the Helsinki Declaration of 1975 as reflected in a priori approval by the institution's human research committee. Every participant signed written informed consent for voluntary participation in the study and prior to sampling for individual genotyping or phenotyping.

### Consent for publication

Not applicable.

## Availability of data and materials

The most important data generated or analysed during this study are included in this published article. Every other, detail, dataset is available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

NT, AO, AM and DP have collected participants' samples and data and have done genotyping and phenotyping analysis. NT also was a major contributor in writing the manuscript. MH, JR and NLK have helped performing of each step of genetic analysis as well as designing the work. NP has done statistical interpretation of data. LP has made substantial contributions to the conception **and** design of the work and also substantively revised it. All authors read and approved the final manuscript.

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