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ORIGINAL ARTICLE

Identification of Gene Candidates Associated with Irritable Bowel Syndrome

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Abstract

Background/Aim: Irritable bowel syndrome (IBS) belongs to the gastrointestinal disorders characterised by abdominal discomfort and pain, altered constipation, diarrhoea and stomach distension. The aim was to assess relationship between the selected genetic polymorphisms with IBS, their combined genotype effect as well as to assess a difference in the distribution of allele and genotype frequencies of selected loci between case and control group.
Methods: This was a prospective study which included 29 participants, 20 individuals diagnosed with IBS based on Rome III criteria and 9 healthy individuals. The study analysed the selected genetic polymorphisms as possible risk factors for IBS according to the model of the case-control study. Genotyping was performed for *FKBP5*, *DRD2* and *DAT* polymorphisms qualified as risk factors for IBS in previous researches.
Results: The results revealed a significant association between DAT polymorphism with IBS, both, at the allelic level (p = 0.006) and genotype level (p = 0.031). Individuals with 434 allelic variant in the genotype have six time higher probability for developing IBS, in comparison to the individuals without this allelic variant. The statistical association between other analysed polymorphism and IBS was not reached. The analysis of combined effects of selected polymorphisms revealed no association with IBS, except *FKBP5* and *DAT* which result was at the level of statistical significance (p = 0.05).
Conclusion: Further analysis which would include *DAT* polymorphism with larger sample size, as well as other genes involved in dopamine neurotransmitter system would be of great interest to define closer conclusion of IBS aetiology.

Key words: Irritable bowel syndrome; Polymorphisms; *FKBP5; DRD2; DAT.*

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Introduction

Irritable bowel syndrome (IBS) belongs to the group of gastrointestinal disorders characterised by abdominal pain, discomfort, diarrhoea and stomach distension, affecting approximately 6 % of population.[1] Its pathophysiology is not completely understood, but its multifactorial basis which includes brain-gut disorders, visceral hypersensitivity, altered microbiota, psychiatric, social and genetic factors are widely accepted as possible causes.[2, 3] Clinical findings in patients with IBS besides gastrointestinal difficulties, includes fatigue, depression and anxiety.[4, 5] Since, no relevant biomarkers for IBS are available, diagnosis is based on the patient's subjective symptoms,

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medical history, endoscopic exams and Bristol stool chart.[6, 7] Due to its heterogenic complexity, many researches are based on the molecular-genetic causes of the disease. For one of the possible causative agent of IBS is considered single nucleotide polymorphism (SNP) within *FKBP5* (FK506 binding protein 5) gene. *FKBP5* is a protein coding gene located on the chromosome 6 at the p21.3 with the role in intracellular glucocorticoid signalling and in increased response to the stress and major depression.[8] Among three described SNPs within the *FKBP5* gene, the most studied is rs1360780, where T allele causes higher values of cortisol in comparison to the carriers of allele C.[9, 10] Thus, previous studies have linked allele T with depression, anxiety, posttraumatic stress syndrome and irritable bowel disease (IBD).[11] The next selected polymorphisms belongs to the dopamine neurotransmitter system, which, together with serotonin neurotransmitter system is considered to play very important role in the aetiology of IBS.[12, 13] The genetic variant in the dopamine neurotransmitter system is *DAT* (dopamine transporter) gene, located on the chromosome 5 at the p15.3. This polymorphic variant is a polymorphism of variable number of tandem repeats (VNTR) with 3-11 repeats, but the most common polymorphisms are 9 (440 bp) and 10 (480 bp). Dopamine relaxes smooth muscles in gastrointestinal tract.[14, 15]

Besides *DAT*, dopamine receptor D2 (*DRD2*), (rs1799732) was another analysed dopamine neurotransmitter gene. *DRD2* encodes D2 subtype of the dopamine receptor and it is located at the on the chromosome 11 at q22-q23.[16] Since physiological function of dopamine is relaying on the action of its receptors, some researches revealed that altered D2 receptor function, by genetic polymorphism in its gene, causes lower levels of dopamine in patients with ulcerative colitis and Crohn's disease compared to the healthy controls.[17] Dysfunction in serotonin and dopamine neurotransmitter system leads to the changes in the bowel motility.[18, 19] These facts were taken into consideration during the selection of polymorphisms in this association-study, according to the case-control model.

The aim of this study was to determine an association between selected polymorphisms, their combined genotype effect with IBS, as well as to assess a difference in the distribution of allele and genotype frequencies of selected loci between case and control group.

Methods

Experiment design, patients and controls

The research represented an association study of selected genetics polymorphisms considered as risk factors for IBS disease. The study included 20 patients diagnosed with IBS according to Rome III criteria[3] and 9 healthy controls. The inclusion criteria for case control group were healthy patients with no IBS symptoms with positive family history of colorectal cancer who went through annual screening colonoscopy. Since patients and controls were recruited at the Clinic, patients with any symptoms of IBS or any other gastrointestinal disorder could not be enrolled in the study as healthy controls.

The patients and controls were selected at the University Clinical Centre of the Republic of Srpska, at the department of Gastroenterology and Hepatology. After confirming voluntary participation in the study by signing previously approved informed consent document, all participants completed standardised clinical questionnaires and provided biological specimens for further stage. Total genomic DNA was isolated from 5 mL of peripheral blood according to Miller.[20] Molecular genetic analysis were performed for three selected genetics polymorphisms which were described as possible risk factors for developing IBS. The selected polymorphisms were within the *FKBP5*, *DRD2* and *DAT* (SLC6A3) genes. The analysis were performed at the laboratory of Human Genetic of Institute for Genetic Engineering and Biotechnology of Sarajevo University.

Molecular Genetic analysis

Molecular Genetics analysis for *FKBP5* genes was performed by allele-specific amplification assay (ASA-PCR), followed by electrophoretic separation on 2 % agarose gel. Primer used for *FKBP5* analysis were F_C: GGC TTT CAC ATA AGC AAA GTT A**C** (Forward primer with **C** at the end, as wild type variant), F_T: GGC TTT CAC ATA AGC AAA GTT A**T** (GGC TTT CAC ATA AGC AAA GTT A**T** with **T** as mutation), R: TGA ATC TGA GAA AGG TTA AGT GG, while expected length of the product were 220 bp. *DRD2* (rs1799732) polymorphism was performed by Restriction Fragment Length Polymorphism (PCR-RFLP) method,[21] followed by electrophoretic separation on 2 % agarose gel. Primers used for this reaction were F: AAA TTT CCA TCT CGG CTC CT and R: GAG GAG CAC CTT CCT GAG TG, while expected

length of products were 300 bp and 160/140 bp. *DAT* polymorphisms was detected by conventional PCR method, followed by sequencing of PCR products on 3500 Genetics Analyzer (Applied Biosystems). Primers used for this amplification were F: 6-FAM-GGT GTA GGG AAC GGC CTG AGA G and R: CTT CCT GGA GGT CAC GGC TCA AGG (471/434 bp). Cycling conditions were: initial denaturation at 95 °C 5 min; denaturation at 95 °C, 30 s; annealing 50 °C, 30 s; elongation 72 °C, 45 s, 40 cycles and final elongation 72 °C, 7 min.

Statistical analysis

The results were expressed numerically or by base pair according to the determined length of selected fragment or as a difference in a nucleotide. For the samples in both examined groups, in which genotyping was performed, the allele and genotype frequencies were determined as well as Hardy-Weinberg equilibrium for all four selected polymorphisms. Statistical package MedCalc 17.4.4 was used to assess associative analysis (Fisher exact test) and χ^2 test to determine an association of selected polymorphisms with occurrence of IBS. Powermarker software was used to assess the association of several combined genotypes with IBS. Significance level was determined as ($p \leq 0.05$).

Results

The study enrolled 29 patients of both genders, 18 (62 %) males and 11 (38 %) females. Of the total number of enrolled patients, 20 were diagnosed with IBS according to Rome Criteria III and 9 patients were controls without any gastrointestinal difficulties.

Genotyping of *FKBP5* (rs1360780) polymorphism was performed for the 19 patients with IBS and 9 controls. The CC (wild type genotype) was not detected in neither examined group, while the frequency of CT genotype was 95 % in patients with IBS and 100 % in the case control group. The frequency of TT genotype was 5 % in patients with IBS, while in case control group TT genotype was not detected. The frequency of allele C was 45 % and 55 % of allele T in the group of patients with IBS, while in case control study the frequency of both allele was 50 %. The Fisher exact test did not reveal statistically significant association of rs1360780 polymorphism with IBS, either at the allelic level ($p = 0.761$) nor at the level of genetic association ($p = 0.548$). The genotype frequency were in Hardy Weinberg equilibrium (HWE).

The genotyping for *DAT* polymorphisms was performed for all 29 samples. The frequency of 472/472 (wild type genotype) was 25 % in the group of patients and 33 % in the case control group. The frequency of 434/434 genotype was 45 % in the group of patients, but this genotype was not detected in the case control group. The frequency of 472 allele was 43.5 % in the group of patients and 83.3 % in the case control group, while the 434 allele was 57.5 % in the patients with IBS and 16.6 % in the case control group. Genotype frequencies of analysed polymorphism were not in HWE (HWE = 0.023). However, the associative analysis revealed that *DAT* polymorphism has strong statistical association with IBS, both, at the allele level ($p = 0.006$) and genotype level ($p = 0.031$), which is presented in Table 1. Individuals with 434 allelic variant in the genotype had six time higher probability for developing IBS, in comparison to the individuals without this allelic variant.

The next analysed polymorphism in *DRD2* gene (rs1799732) was genotyped for all 29 samples and expected fragments were 300 bp and 160/140 bp. The frequency of 300/300 was 5 % in the group of patients, but it was not detected in the group of controls. The frequency of 300/160/140 genotype was 30 % in the group of patients and 11 % in the group of controls. However, the frequency of 160/140/160/140 (wild type) genotype was 65 % in the group of patients and 88 % in the group of controls. The frequency of 300 allele was 20 % in the group of patients and 5 % in control group, while frequency of 160/140 allele was 80 % in the group of patients and 94 % in the control group. The frequency of allele 300 was 20 % in the group of patients and 5 % in the case control group, while the frequency of 160/140 allele was 80 % in the group of patients and 94 % in the case control group. Genotype frequencies of

Table 1: Association of alelle and genotypes of FKBP5, DRD2 and DAT polymorpishms with IBS and HWE

Polymorphism	Allele Exact p-value	Genotype Exact p-value	HWE
FKBP5	0.610	0.548	0.000
DRD2	0.251	0.568	0.512
DAT	**0.006**	**0.031**	0.023

FKBP5- FK506 binding protein 5; DRD2- Dopamine receptor D2 ; DAT- dopamine transporter; HWE- Hardy-Weinberg equilibrium; IBS- Irritable bowel syndrome;

Table 2: Combined effects of polymorphisms with IBS

Combined effects of polymorphisms	p-value
DRD2-FKBP5	0.2678
FKBP5-DAT	0.0535
DRD2-FKBP5-DAT	0.1945

FKBP5- FK506 binding protein 5; DRD2- Dopamine receptor D2 ; DAT- dopamine transporter; IBS-Irritable bowel syndrome;

analysed polymorphism were not in equilibrium (HWE = 0.5120). No statistical association with IBS was detected neither at the allelic (p = 0.251), nor genotype level (p = 0.568).

The analysis of combined effects of 2, 3 and 4 polymorphic variants was did not reveal statistically significant association with IBS, however, the combination of *FKBP* and *DAT1* polymorphism was at the level of statistical significance (p = 0.05), which is presented in Table 2.

Discussion

IBS is biopsychosocial disorder which appearance is characterised by several factors including intestinal motility disorders, gastrointestinal sensational abnormalities, various intestinal inflammations and infections, psychological and other affective disorders.[2, 22] Due to its heterogenic and multifactorial basis, this study aimed to analyse several selected polymorphism which are possible biomarkers for IBS. The study of Camileri et al, analysed 4456 polymorphism of which 12 were within the *FKBP5* gene.[23] Their study revealed that 2 out of 12 were significant after Bonferroni correction. In this research *FKBP5* gene (rs1360780) analysed by the Fisher exact test did not reveal a statistically significant association with IBS, neither at the allelic nor at the genotype level. The genotype frequencies were in HWE, thus the obtained result can be considered as valid. Higher level of cortisol are characteristic for the carriers of T allele,[9] in comparison to C allele (wild type), while genotype results from presented study showed that only one sample (P019) was homozygote (TT genotype), while the rest of the patients had CT genotype. In the case control group, all patients were heterozygote carriers. The sample P019 is a patients with subtype IBS-D, even though was the only detected case, further research could be focused on the analysis of this SNP with specific diarrhoea predominant subtype of IBS, in order to make more precise conclusion about its eventual connection. On the other side, some researches showed an association of altered dopamine receptor D2, caused by SNP (rs1799732) with various type psychotic disorders, overweight, obesity and hedonic hunger.[24] Also, deletion of C allele within this polymorphism causes lower level of dopamine which can be bounded to its receptors, thus causing lower levels of this hormone in patients with ulcerous colitis and Crohn's disease compared to the healthy controls.[17] Besides dopamine receptor D2, dopamine transporter *DAT* is another gene with possible role in IBS.[25] Since dopamine and serotonin transporters have an important role in activation of muscle contractions and colon motility[10] it was aimed to investigate the role VNTR in *DAT* gene with IBS. Results revealed no statistical association at the allelic and genotype level between *DRD2* and IBS. Genotype frequencies for *DRD2* polymorphisms were not in HWE. Also, curtain deviation from equilibrium were noticed in the genotype frequencies for *DAT* polymorphism. The deviation can be explained as a real association of this genetic marker with the analysed trait, as well as due to the small sample size. Analysing *DAT* polymorphism, the strong association with IBS was found at the allelic (p = 0.006) and genotype (p = 0.023) level, which leads to the conclusion that *DAT* polymorphism could be associated with IBS in the analysed group of patients. To the authors' knowledge, this was the first study investigating an association of selected polymorphism with IBS. However, the combined effects of these polymorphism showed no statistically significant association with IBS.

Conclusion

This study represents very important step in identification of gene candidates and theirs allelic variants as possible causes of IBS, as well as for better understanding this phenotypically unusable and aetiologically complex disease. However, the study was limited with the number of patients enrolled in the research, particularly healthy controls, since not many patients with positive family history of colorectal cancer are aware of preventive colonoscopies, which was the main reason to visit the specialist. Besides larger sample size, more gene candidates should be examined in order to define more precise conclusion. Since IBS is very heterogeneous disorder many different factors are participating in its ethology which should be also taken into consideration.

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None.

Conflict of interest

None.

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