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ORIGINAL ARTICLE

CFTR IVS8 Poly-T Variation Affects Severity of Acute Pancreatitis in Women

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Abstract

Background Cystic fibrosis transmembrane conductance regulator (CFTR) is important for normal pancreatic function. Its coding gene is polymorphic, and the variations have been associated with the increased risk for acute pancreatitis. However, their impact on the disease severity is still unknown. Therefore, the aim of our study was to determine the functional importance of common cystic fibrosis transmembrane conductance regulator variations IVS8-poly T, R117H, and M470V for the severity of acute pancreatitis.

Method The study involved 98 acute pancreatitis patients. The severity of the disease was determined based on the Atlanta Classification system. IVS8-poly T, R117H, and M470V genotyping was performed using PCR-RFLP method.

Results IVS8-5T, IVS8-7T, IVS8-9T, and M470V alleles were found at the frequencies of 5.7, 75.5, 18.9, and 55.7%, respectively, while R117H was not observed. Among women, the severe form of the disease was more frequent in carriers of at least one IVS8 9T allele (RR for 9T/9T + 9T/non-9T vs. non-9T/non-9T: 2.115; 95% CI: 1.241–3.605). This association was not detected in men and was not affected by M470V. In addition, co-morbidities increased the severity of acute pancreatitis ($p = 0.022$).

Conclusion Our study reveals that IVS8 poly-T variation affects severity of acute pancreatitis in women and that existent co-morbidities worsen the clinical course of the disease.

Keywords Acute pancreatitis · CFTR · IVS8-poly T · Sex

Introduction

Acute pancreatitis is a gastrointestinal disease characterized by acute onset of pancreatic inflammation. Its incidence is increasing worldwide, and the mortality, depending on the severity of the disease, could be as high as 50%.[1] The development and the course of acute pancreatitis are both affected by numerous factors, including the alteration of pancreatic proteins that are important for normal function of the gland.[2]

Cystic fibrosis transmembrane conductance regulator (CFTR) is one of the pancreatic proteins, involved in pancreatic juice production. It consists of 1480 amino acids and serves as an anion transmembrane channel for Cl^- and $HCO3^-$. In exocrine pancreas, it transports bicarbonate from the pancreatic duct cell to the lumen of the duct, which in turn pulls water and increases the volume, and therefore the flow, of pancreatic juice. When its function is diminished or missing, the volume of pancreatic juice is reduced and the density is increased; the pancreatic duct is more prone to obstruction, proenzymes are prematurely activated, and pancreatitis develops.[3]

CFTR gene is located on a long arm of chromosome 7 (q31-q32) and consists of 250-kb-long genomic sequence, which includes 27 exons. It is extremely polymorphic, with more than 2000 variations described so far (http://www.genet.sickkids.on.ca). According to the molecular defect and its phenotypic consequences, *CFTR* variations have been classified into six groups.[3] To assess the risk of pancreatitis

The study was approved on August 29th, 2011, by the ethics committee at the Clinical Centre Kragujevac, decision No 01-9024. The study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions, with all the patients giving their written informed consent.

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in carriers of *CFTR* variations, a Pancreatic Insufficiency Prevalence (PIP) scoring system was also developed, classifying them based on functional severity as mild (< 0.25) or severe (> 0.25).[4]

Previous study with cystic fibrosis patients showed an increased risk of pancreatitis in mild *CFTR* variation carriers compared to those carrying severe variations.[4] The observed association suggests that the certain level of pancreatic acinus reserve is necessary for the onset of pancreatitis, stressing the role of seemingly less important variations in the disease development. However, as to our best knowledge, there are no studies evaluating the impact of *CFTR* genetics on the course of already developed disease. The aim of our study was to determine the functional importance of three of the most commonly tested mild *CFTR* variations, namely IVS8-poly T, R117H, and M470V,[3] for the severity of acute pancreatitis.

Materials and Methods

Ninety eight acute pancreatitis patients, hospitalized between November 2011 and May 2014 at the Intensive Care Unit, Clinical Centre Kragujevac, Serbia, participated in the study. The diagnosis has been established based on the presence of at least two of the following three features: (1) abdominal pain characteristic of acute pancreatitis, (2) at least 3-fold increase of serum amylase and/or lipase, and (3) characteristic computed tomography findings. The severity of the disease was determined based on the Atlanta Classification system, i.e., minimal organ dysfunction and uneventful recovery defined mild form, while local complications (necrosis, abscess, pseudocyst) and/or organ failure were interpreted as characteristics of severe acute pancreatitis. The study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions, with all the patients giving their written informed consent. The study was approved on August 29th, 2011, by the ethics committee at the Clinical Centre Kragujevac, decision No 01-9024.

Genomic DNA was isolated from EDTA blood samples using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). *CFTR* genotyping was performed using PCR-RFLP method, according to Shrimpton et al.[5] for IVS8 poly-T (rs1805177, 5T/7T/9T allele) and 350G>A (rs78655421, R117H) and to Qiao et al.[6] for 1408G>A (rs213950, M470V), with slight modifications of PCR reagents and conditions (available upon request). All PCR reactions were performed on Techne Genius PCR Thermal Cycler (Techne, Cambridge, UK), and the reagents were purchased from Invitrogen (Carlsbad, CA), New England Biolab (Ipswich, MA), or Thermo Scientific (Waltham, MA). The PCR amplicons and restriction fragments were detected by gel electrophoresis on 1.2 or 2.4% agarose gel stained with Sybr® safe DNA gel stain (Invitrogen, Carlsbad, CA).

Statistical analyses were performed with SPSS Statistics, version 20 (IBM, Armonk, NY, USA). Haplotype analysis and haplotype frequency calculations were carried out using the population genetic software program Arlequin, version 3.11 (http://cmpg.unibe.ch/software/arlequin3). Genotype data were presented as allele, genotype, haplotype, and diplotype frequencies. The Hardy-Weinberg equilibrium was tested using Chi-square statistics. Two-tailed Fisher exact, Fisher-Freeman-Halton, Pearson Chi-square test, and logistic regression analysis were used to compare the frequencies of alleles, genotypes, diplotypes, and genotype groups (determined using dominant or recessive genetic model), as well as other patient characteristics (age, sex, co-morbidities, etiology of pancreatitis, alcohol consumption, cigarette smoking) between mild and severe acute pancreatitis patients. The association between *CFTR* genotype and the severity of acute pancreatitis was assessed by estimating the relative risk (RR) of complications and/or organ failure with 95% confidence intervals (95% CI). *P* value less than 0.05 was considered significant.

Results

There were 38 women and 60 men enrolled in the study, with median age of 61 (range from 23 to 86), and the body mass index from 19.2 to 42.2 (median value of 27.2). Of them, 53 (17 women) and 45 (21 women) were classified as mild and severe acute pancreatitis patients, respectively. Etiology of pancreatitis has been confirmed as alcoholic in 22 patients (3 women), biliary in 51 (28 women), and idiopathic in other 25 (5 women). As expected, the most frequent type of acute pancreatitis in women was biliary ($\chi^2 = 15.3$, $p < 0.0001$).

All *CFTR* genotype frequencies were in accordance with Hardy-Weinberg equilibrium ($\chi^2 < 3.346$, $p = 0.05$). *CFTR* 350G>A variation (R117H) was not observed. Based on the length of the IVS8 poly-T residue, two stratification strategies were employed: subjects were assigned to carriers or non-carriers of (a) the shortest (5T vs. non-5T) and (b) the longest (9T vs. non-9T) polythymidine tract. Genotype groups were determined using both dominant and recessive genetic models (comparing homozygous carriers of the wild-type allele with carriers of at least one variant allele and carriers of at least one wild-type allele with homozygous carriers of the variant allele, respectively), but no significant difference in their frequency was observed between mild and severe acute pancreatitis patients. The frequency distributions of *CFTR* alleles, genotypes, and genotype groups according to the severity of acute pancreatitis are presented in Table 1. The frequencies of estimated haplotypes and diplotypes did not differ significantly between the groups (Table 2). Of other examined variables, i.e., patients' age, sex, existing co-morbidities (expressed by Charlson Comorbidity Index, CCI), etiology of pancreatitis,

Table 1 *CFTR* allele, genotype, and genotype group frequencies in patients with mild ($n = 53$) and severe ($n = 45$) acute pancreatitis

	Mild acute pancreatitis	Severe acute pancreatitis	*P*
Allele			
rs1805177 (IVS8 poly-T)			
IVS8 5T	0.057 (6/106)	0.022 (2/90)	0.211
IVS8 7T	0.755 (80/106)	0.700 (63/90)	
IVS8 9T	0.189 (20/106)	0.278 (25/90)	
IVS8 non-5T	0.943 (100/106)	0.978 (88/90)	0.225
IVS8 non-9T	0.811 (86/106)	0.722 (65/90)	0.139
rs78655421 (350G>A, R117H)			
350G	1.000 (106/106)	1.000 (90/90)	1.000
350A	0.000 (0/106)	0.000 (0/90)	
rs213950 (1408G>A, M470V)			
1408G	0.443 (47/106)	0.478 (43/90)	0.630
1408A	0.557 (59/106)	0.522 (47/90)	
Genotype			
rs1805177 (IVS8 poly-T)			
5T/5T	0.000 (0/53)	0.000 (0/45)	0.370
5T/7T	0.038 (2/53)	0.022 (1/45)	
5T/9T	0.075 (4/53)	0.022 (1/45)	
7T/7T	0.660 (35/53)	0.556 (25/45)	
7T/9T	0.151 (8/53)	0.267 (12/45)	
9T/9T	0.075 (4/53)	0.133 (6/45)	
5T/non-5T	0.113 (6/53)	0.044 (2/45)	0.282
non-5T/non-5T	0.887 (47/53)	0.956 (43/45)	
9T/non-9T	0.226 (12/53)	0.289 (13/45)	0.411
non-9T/non-9T	0.698 (37/53)	0.578 (26/45)	
rs78655421 (350G>A, R117H)			
G/G	1.000 (53/53)	1.000 (45/45)	1.000
G/A	0.000 (0/53)	0.000 (0/45)	
A/A	0.000 (0/53)	0.000 (0/45)	
rs213950 (1408G>A, M470V)			
G/G	0.189 (10/53)	0.222 (10/45)	0.896
G/A	0.509 (27/53)	0.511 (23/45)	
A/A	0.302 (16/53)	0.267 (12/45)	
Genotype group			
Dominant genetic model			
rs1805177 (IVS8 poly-T)			
5T/non-5T + non-5T/non-5T	1.000 (53/53)	1.000 (45/45)	NA
9T/non-9T + non-9T/non-9T	0.925 (49/53)	0.867 (39/45)	0.505
rs213950 (1408G>A, M470V)			
G/A + A/A	0.811 (43/53)	0.778 (35/45)	0.803
Recessive genetic model			
rs1805177 (IVS8 poly-T)			
5T/5T + 5T/non-5T	0.113 (6/53)	0.044 (2/45)	0.282
9T/9T + 9T/non-9T	0.302 (16/53)	0.422 (19/45)	0.290
rs213950 (1408G>A, M470V)			
G/G + G/A	0.698 (37/53)	0.733 (33/45)	0.823

alcohol consumption, and cigarette smoking, only CCI was found to be associated with increased severity of acute pancreatitis ($p = 0.022$).

The possible effect of the length of the IVS8 poly-T residue on the severity of acute pancreatitis was tested in men and women separately, with the frequency distribution of alleles,

Table 2 *CFTR* haplotype and diplotype frequencies in patients with mild ($n = 53$) and severe ($n = 45$) acute pancreatitis

	Mild acute pancreatitis	Severe acute pancreatitis	*P*
Haplotype			
rs1805177-rs78655421-rs213950			
5T-350G-1408A	0.019 (2/106)	0.000 (0/90)	0.554
5T-350G-1408G	0.038 (4/106)	0.022 (2/90)	
7T-350G-1408A	0.500 (53/106)	0.467 (42/90)	
7T-350G-1408G	0.255 (27/106)	0.233 (21/90)	
9T-350G-1408A	0.038 (4/106)	0.056 (5/90)	
9T-350G-1408G	0.151 (16/106)	0.222 (20/90)	
Diplotype			
rs1805177-rs78655421-rs213950			
5T-350G-1408A/9T-350G-1408A	0.019 (1/53)	0.000 (0/45)	0.739
5T-350G-1408G/7T-350G-1408A	0.019 (1/53)	0.000 (0/45)	
5T-350G-1408G/7T-350G-1408G	0.019 (1/53)	0.022 (1/45)	
5T-350G-1408G/9T-350G-1408G	0.038 (2/53)	0.022 (1/45)	
7T-350G-1408A/7T-350G-1408A	0.283 (15/53)	0.222 (10/45)	
7T-350G-1408A/9T-350G-1408A	0.000 (0/53)	0.022 (1/45)	
7T-350G-1408G/7T-350G-1408A	0.283 (15/53)	0.289 (13/45)	
7T-350G-1408G/7T-350G-1408G	0.094 (5/53)	0.044 (2/45)	
7T-350G-1408G/9T-350G-1408G	0.019 (1/53)	0.067 (3/45)	
9T-350G-1408A/9T-350G-1408A	0.00 (0/53)	0.022 (1/45)	
9T-350G-1408G/5T-350G-1408A	0.019 (1/53)	0.000 (0/45)	
9T-350G-1408G/7T-350G-1408A	0.132 (7/53)	0.178 (8/45)	
9T-350G-1408G/9T-350G-1408A	0.057 (3/53)	0.044 (2/45)	

genotypes, and genotype groups presented in Table 3. The results revealed that in female patients with acute pancreatitis, the risk of developing the severe form of the disease is more than two times higher if she is a carrier of at least one *CFTR* IVS8 9T allele (RR for 9T/9T + 9T/non-9T vs. non-9T/non-9T: 2.115; 95% CI: 1.241–3.605). Additional haplotype and diplotype-based comparison showed that female carriers of at least one 9T-350G-1408G haplotype were significantly more frequent among severe acute pancreatitis patients ($p = 0.021$). This association was not detected in men (RR for 9T/9T + 9T/non-9T vs. non-9T/non-9T: 1.158; 95% CI: 0.594–2.256) and was not affected by 1408G>A (M470V) genotype ($p = 0.545$). The association of *CFTR* IVS8 9T allele and severity of acute pancreatitis with biliary etiology (the most frequent type in women) was not observed (RR for 9T/9T + 9T/non-9T vs. non-9T/non-9T: 1.123; 95% CI: 0.709–1.778). The significance of IVS8 poly-T variation for severity of acute pancreatitis in women was confirmed by both univariate ($p = 0.015$) and multivariate ($p = 0.030$) logistic regression, while the role of other examined variables was not detected ($p = 0.308$). The best fitting model, which included genotype, patients' age, and existing co-morbidities (Cox & Snell R^2: 0.252, Nagelkerke R^2: 0.337, Hosmer-Lemeshow $\chi^2 = 4.642$, $df = 8$, $p = 0.795$), confirmed the recessive effect of *CFTR* IVS8 9T allele on acute pancreatitis severity in women ($p = 0.012$; Table 4).

Discussion

The main finding of our study is that in women with acute pancreatitis, the risk of developing the severe form of disease is more than two times higher if she is a carrier of at least one *CFTR* IVS8 9T allele. As to our best knowledge, this is the first study to report this finding. In addition, in acute pancreatitis patients, CCI scores were positively associated with the disease severity.

It is well known that reduced $HCO3^-$ secretion in pancreatic duct contributes to the development of acute pancreatitis. Namely, to maintain normal functioning of pancreas, acinar and ductal cells daily secrete up to 2 l of alkaline juice.[7] While secretion from acinar cells results in a small amount of NaCl-rich fluid, ductal cells increase the volume of pancreatic juice by exchanging chloride ions from the lumen for bicarbonates and consequently pulling water into the lumen.[7] Since extracellular acidosis promotes pancreatitis development by triggering activation of proenzymes in pancreatic acini and duct,[8] efficient bicarbonate secretion is very important in preventing the onset of the disease. The described anion exchange, which is crucial both for neutralization of the secreted fluid and for the duct flushing, largely depends on CFTR transporting activity.[7]

CFTR is an ATP-binding cassette (ABC) transporter that acts as an anion channel.[7] It consists of one regulatory domain

Table 3 *CFTR* IVS8 poly-T allele, genotype, and genotype group frequencies in male and female acute pancreatitis patients

	Men ($n = 60$)			Women ($n = 38$)		
	Mild acute pancreatitis	Severe acute pancreatitis	p	Mild acute pancreatitis	Severe acute pancreatitis	p
Allele						
IVS8 5T	0.083 (6/72)	0.042 (2/48)	0.587	0.000 (0/34)	0.000 (0/42)	0.017
IVS8 7T	0.694 (50/72)	0.771 (37/48)		0.882 (30/34)	0.619 (26/42)	
IVS8 9T	0.222 (16/72)	0.188 (9/48)		0.118 (4/34)	0.381 (16/42)	
Genotype						
5T/5T	0.000 (0/36)	0.000 (0/24)	0.954	0.000 (0/17)	0.000 (0/21)	0.017
5T/7T	0.056 (2/36)	0.042 (1/24)		0.000 (0/17)	0.000 (0/21)	
5T/9T	0.111 (4/36)	0.042 (1/24)		0.000 (0/17)	0.000 (0/21)	
7T/7T	0.565 (20/36)	0.625 (15/24)		0.882 (15/17)	0.476 (10/21)	
7T/9T	0.222 (8/36)	0.250 (6/24)		0.000 (0/17)	0.286 (6/21)	
9T/9T	0.056 (2/36)	0.042 (1/24)		0.118 (2/17)	0.238 (5/21)	
5T/non-5T	0.167 (6/36)	0.083 (2/24)	0.457	0.000 (0/17)	0.000 (0/21)	NA
Non-5T/non-5T	0.833 (30/36)	0.917 (22/24)		1.000 (17/17)	1.000 (21/21)	
9T/non-9T	0.333 (12/36)	0.292 (7/24)	0.902	0.000 (0/17)	0.286 (6/17)	0.017
Non-9T/non-9T	0.611 (22/36)	0.667 (16/24)		0.882 (15/17)	0.476 (10/17)	
Genotype group						
Dominant genetic model						
5T/non-5T + non-5T/non-5T	1.000 (36/36)	1.000 (24/24)	NA	1.000 (17/17)	1.000 (21/21)	NA
9T/non-9T + non-9T/non-9T	0.944 (34/36)	0.958 (23/24)	1.000	0.882 (15/17)	0.762 (16/21)	0.427
Recessive genetic model						
5T/5T + 5T/non-5T	0.167 (6/36)	0.083 (2/24)	0.457	0.000 (0/17)	0.000 (0/21)	NA
9T/9T + 9T/non-9T	0.389 (14/36)	0.333 (8/24)	0.787	0.118 (2/17)	0.524 (11/21)	0.015

(RD) that connects two homologous halves, each containing two cytoplasmic nucleotide binding domains (NBD1 and NBD2), coupled with corresponding pore-forming membrane spanning domains MSD1 and MSD2.[9] The channel opening and closing relies on an ATP-driven CFTR conformation change, enabled by phosphorylation at several sites within RD.[10] In brief, binding of ATP initiates NBD1-NBD2 dimerisation and subsequent MSDs outward-facing conformation, which leads to channel opening.[11] In contrast, ATP hydrolysis reverses the process and closes the channel.[12] Based on the previous observations on the role of NDB1 and NBD2 in CFTR gating,[11] one might expect that the loss or alteration of any of the NBDs could result in decreased CFTR function.

Of all genes mechanistically linked to pancreatic function, *CFTR* seems to be the most variable. One of the most frequently studied *CFTR* variation is IVS8-poly T, which represents an intronic sequence composed of five, seven, or nine thymidines. It has been demonstrated that the shorter poly-T sequence often affects splicing of exon 9, leading to synthesis of truncated NBD1.[13] Due to the importance of NBD1 for channel gating, CFTR is more efficient if this poly-T tract is longer, and this has been confirmed by several studies.[5] Therefore, the possibility that the *CFTR* IVS8 9T results in CFTR hypofunction seems paradoxical and unlikely.

Previous investigations showed that the described IVS8 poly-T effect might depend on simultaneous presence of another *CFTR* variation on the same chromosome, namely R117H.[5] R117H represents a missense mutation that affects the structure of the first extracellular loop of MSD1, causing decreased CFTR function by reducing number of channels, channel conductance, and average open probability.[14] As in

Table 4 Summary of variable estimates using multiple logistic regression analysis regarding severity of acute pancreatitis in women ($n = 38$)

Variables	β	SE	Wald χ^2	p	OR	95% CI
Recessive *CFTR IVS8 9T* genetic model	2.533	1.009	6.298	0.012	12.594	1.741–91.074
Charlson Comorbidity Index	0.294	0.418	0.495	0.482	1.342	0.591–3.044
Age	0.040	0.030	1.791	0.181	1.041	0.982–1.104

β the regression coefficient, *SE* the standard error of β, *Wald* χ^2 Wald test statistic for DF = 1, p the probability value, *OR* odds ratio, *95% CI* the 95% confidence interval for the estimated OR

our study, none of the acute pancreatitis patients was a carrier of R117H, the effect of *CFTR* IVS8 9T that we observed seems independent of R117H. The association between 9T allele and severity of acute pancreatitis was not affected by M470V either, although the latter decreases channel activity[13] and has been previously linked to IVS8-poly T variation.[15] Still, none of this explains the apparent paradox related to the observed *CFTR* IVS8 9T effect.

In attempt to understand the mechanism behind our findings, we have developed several theories. Firstly, in severe acute pancreatitis, the most common organ failure is respiratory, and it develops due to accumulation of fluid between the alveolar membrane and the capillaries in the lung.[16] The fluid component of the secretion in lungs, i.e., in the submucosal glands of distal tubules and acini, depends on transporting activity of CFTR.[7] Therefore, in the presence of already existing acute pancreatitis, the carriers of fully functional *CFTR* IVS8 9T allele could more easily develop pulmonary edema, resulting in respiratory organ failure that defines pancreatic disease as severe. However, this explanation does not clarify why the observed *CFTR* IVS8 9T effect would be present only in women.

On the other hand, sex specificity of genetics' influence on disease prevalence and course has been already recognized. Well-known examples of this sexual dimorphism include association between *ACE* gene variation and hypertension, observed only in men,[17] or between *RELN* gene variation and schizophrenia, detected only in women.[18] Similar has been reported for *CFTR* IVS8-poly T in relation to cystic fibrosis, 5T variant apparently having milder consequences in women compared to men.[19] Low penetrance of 5T allele, observed in women with a CFTR-related disease such as cystic fibrosis, might be an explanation for severe clinical course in female acute pancreatitis patients carrying 9T allele.

In addition to cis-acting elements (such as R117H) and epigenetic regulation, sex-specific gene expression and penetrance could be influenced by non-genetic factors too, including environmental and hormonal influences.[20] In acute pancreatitis, environment has an important role in both etiology and clinical course of the disease.[21] The environmental effects are also sex-specific, with the two most frequent types of pancreatitis, namely alcoholic and biliary, predominating in men and women, respectively.[22] It has been observed that in alcohol-induced pancreatitis, dominant type of acinar cell death is necrosis,[23] while the bile salts, present in excess in acinus lumen in biliary pancreatitis, could activate both apoptosis and necrosis.[24] In addition, the type of pancreatic acinar cell death has been associated with the severity of acute pancreatitis too, mild form usually coupled with apoptosis, and severe with necrosis.[25] As in the presence of CFTR dysfunction sensitivity of cells to apoptogenic agents is increased,[26] it can be expected that women with fully functional CFTR and biliary etiology of acute pancreatitis would be more prone to necrosis of acinar cells, thus more easily develop severe form of the disease. However, in our study, sample etiology was not associated with increased severity of acute pancreatitis.

Nevertheless, acute pancreatitis development and course might also be affected by sex hormones: estrogens seem to alter pancreatic function by inhibiting CFTR and decreasing bicarbonate production.[27] On the other hand, CFTR itself regulates synthesis of estrogens by turning up the signal of FSH-stimulated estrogen production.[28] We believe that this negative feedback loop between estrogen and CFTR might be the most plausible explanation for our main finding. Namely, female carriers of fully functional IVS8 9T allele (as compared to female IVS8 5T or 7T carriers) would have higher levels of estrogens, which in turn would inhibit CFTR function in other organs, including pancreas. Reduced bicarbonate secretion in pancreatic duct would then increase the risk for development or (if the disease is already present) the severity of acute pancreatitis. As healthy subjects did not participate in our study, we were not able to estimate the influence of investigated factors on the prevalence of acute pancreatitis. Yet, we did observe different frequency distribution of IVS8 poly-T alleles in Serbian acute pancreatitis patients compared to healthy Serbian volunteers,[29] implicating the possibility of association between this variation and the disease development.

The Charlson Comorbidity Index (CCI) was introduced in 1987 as a tool for prediction of the 10-year mortality for patients with co-morbidities that are enrolled in longitudinal studies. Earlier studies on acute pancreatitis revealed that co-morbidity does contribute to organ failure and mortality.[30] In the present study, in line with the previous reports, higher CCI was associated with increased severity of acute pancreatitis.

Conclusion

Our study reveals that *CFTR* IVS8 poly-T variation affects severity of acute pancreatitis in women and that existent co-morbidities worsen the clinical course of the disease. To confirm these findings and test their potential clinical applicability, additional studies on larger and ethnically diverse population, with inclusion of other genetic and non-genetic factors, are warranted.

Author's Contribution Ivan Radosavljevic contributed acquisition of data, interpretation of data, and drafting of the manuscript. Bojan Stojanovic and Marko Spasic contributed acquisition of data. Slobodan Jankovic contributed study concept and design and interpretation of the data. Natasa Djordjevic contributed study concept and design, statistical analysis, interpretation of the data, and drafting of the manuscript. All authors contributed to critical manuscript revision.

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Compliance with Ethical Standards

The study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions, with all the patients giving their written informed consent. The study was approved on August 29th, 2011, by the ethics committee at the Clinical Centre Kragujevac, decision No 01-9024.

Conflict of Interest The authors declare that they have no conflict of interest.

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