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Estimating the mode of inheritance of edematous pancreatitis based on genes CFTR (rs 113993960), IL-4 (rs 2243250), PRSS1 (rs 111033565), SPINK1 (rs ID6690) and TNF-α (rs 1800629) polymorphism

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Abstract
The occurrence of mutations of genes such as cystic fibrosis gene (CFTR - cystic fibrosis transmembrane conductance regulator), cationic trypsinogen gene (PRSS1) and pancreatic secretory trypsin inhibitor (SPINK1), which influence the acute pancreatitis or chronic pancreatitis phenotype formation, substantially differs in different populations and ethnic groups, it also associates with hereditary (family) factor. The aim of the research was to estimate the mode of inheritance of edematous pancreatitis based on genes CFTR (rs 113993960), IL-4 (rs 2243250), PRSS1 (rs 111033565), SPINK1 (rs ID6690) and TNF-α (rs 1800629) polymorphism. The study involved 123 patients with acute and chronic pancreatitis exacerbation. The molecular genetic studies included the determining of polymorphic variants of genes IL-4 (C-590T), TNF-α (G-308A), PRSS1 (R122H), SPINK1 (N34S) and CFTR (delF508). The possible inheritance models of edematous pancreatitis were analysed using logistic regression. The analysis of the inheritance models of edematous pancreatitis taking into consideration polymorphic variants of gene *IL-4* (rs 2243250) found that the recessive model has higher chances to manifest in the presence of the *TT*-genotype of gene *IL-4*, than the *C*-allele (OR=1.21; 95% CI: 0.34-5.66, $p>0.05$). *TT*-genotype of gene *IL-4* increases chronic pancreatitis exacerbation risk, almost doubles it (OR=1.93; $p>0.05$), whereas the *C*-allele carrier makes the chances of this one credibly the lowest in the investigated population (OR=0.41, 95%CI OR: 0.16-1.03; p=0.046). The genes *PRSS1 (365G>A), CFTR (delF508), SPINK1 (215G-A)* and *TNF-α (G-308A)* polymorphism is not the risk factor of the appearance of edematous pancreatitis, neither of alcoholic nor of biliary origin. The analysis of the inheritance models of edematous pancreatitis, taking into consideration polymorphic variants of gene *IL-4* (rs 2243250), found that the most effective is recessive model. The *C*-allele carrier is protective.

Keywords: Alcoholic, biliary, genotype, pancreatitis, polymorphism, CFTR (ΔF508), PRSS1 (R122H), IL-4 (C-590T), TNF-α (G-308A)

1. Introduction
Genetic association studies, candidate-gene and genome-wide association studies assess the association between a disease and genetic variants (gene polymorphisms) in a population. The mutations in genes such as cystic fibrosis gene (CFTR - cystic fibrosis transmembrane conductance regulator), cationic trypsinogen gene (PRSS1) and pancreatic secretory trypsin inhibitor (SPINK1) were often studied in association with pancreatitis [1, 2, 3, 4, 5]. The occurrence of above mentioned genetic mutations, which influence the acute pancreatitis (AP) or chronic pancreatitis (CP) phenotype formation, substantially differs in different populations and ethnic groups, it also associates with hereditary (family) factor [6, 7, 8, 9, 10, 11].
It should be noted that the role of immune system in the pathogenesis of AP and exacerbation of CP (ECP) leaves unattended of investigators, particularly from the stand of the influence of polymorphisms of genes that regulate the inflammatory response (interleukin -1β (IL-1β), -4 (IL- 4), -6 (IL-6) and tumour necrosis factor-alpha (TNF-α), etc.) and the clinical course of pancreatitis [12].
In addition, the studies of the same gene polymorphisms substantially differ in different populations and ethnic groups, and expressions of these mutations depend on the combination of cultural, social, economic and environmental factors [13- 21].
Based on the above, it was necessary to study the structure of the mutations of genes CFTR (rs 113993960), IL-4 (rs 2243250), PRSS1 (rs 111033565), SPINK1 (rs ID6690) and TNF-α (rs 1800629) and their inheritance models in the North Bukovina population to establish their role in the pathogenesis of acute edematous pancreatitis with the purpose to identify regularities

and mechanisms of AP and ECP formation, the selection of high-risk groups, to do prognosis and prevention.
The research of this genes combination of the pancreas pathology was not conducted in Ukraine before the beginning of this study. However, some of individual not numerous studies of mononucleotide polymorphisms of genes PRSS1 and SPINK1 were started during the last five years in Ukraine (Chernivtsi) for AP, mostly its destructive forms [22] and did not consist of CP. Also no inheritance models of edematous pancreatitis were studied in Ukraine at all.

1.1 The aim of the research: To estimate the mode of inheritance of edematous pancreatitis and the risk of its appearance based on genes CFTR (rs 113993960), IL-4 (rs 2243250), PRSS1 (rs 111033565), SPINK1 (rs ID6690) and TNF-α (rs 1800629) polymorphism.

2. Materials and methods

2.1 Compliance with bioethics

Study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics of the Ministry of Health of Ukraine. Patients' Examination Cards and Patients' Informed Consent Forms were approved by the Biomedical Ethics Commission of Bukovina State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). All enrolled patients were treated in the Local Emergency hospital (Chernivtsi, Ukraine). Genetic bench study performed at the laboratory of the State institution «Reference centre of molecular diagnostics of the Ministry of Health of Ukraine» (Kyiv) and at the laboratory of Medical Biology and Genetics Department of Bukovina State Medical University. After screening (matching inclusion/exclusion criteria) 123 patients with exacerbation of chronic pancreatitis (ECP) and AP (edematous form) were selected for further examination. The control group included 40 practically healthy individuals who were not relatives of the patients, without reliable differences of sex and age.

2.2 Diagnosis of Acute Pancreatitis and Exacerbation of Chronic Pancreatitis.

The diagnosis of AP and ECP was made on the basis of the existing national and international recommendations on the diagnosis and treatment of acute pancreatitis [23, 24]. All patients signed an informed consent to participate in the research and they underwent a complex of examinations: clinical, laboratory and instrumental ones according to protocol recommendations.

2.3 Genotyping

The polymorphic variants of analysed genes IL-4 (C-590T), TNF-α (G-308A), PRSS1 (R122H), SPINK1 (N34S) and CFTR (delF508) were studied by polymerase chain reaction (PCR) method using oligonucleotide primers of the company «Metabion» (Germany) according to the modified protocols [25-28]. The amplification products of DNA fragments of gene were further digested with restriction endonuclease ("Thermo Scientific", USA): enzyme PmlI (Eco72I) for gene PRSS1, AvaII - for gene IL-4, NcoI - for gene TNFα, *Pst*I ("Fermentas®", Germany) – for gene SPINK1. The received fragments were analysed by agarose gel electrophoresis and stained with ethidium bromide, molecular weight marker GeneRuler 50 bp (DNA Ladder, "Thermo Scientific", USA), with further visualization by using transilluminator and Vitran software.

2.4 The genotypes distribution with gene polymorphism assessment

The correspondence of the genotypes distribution with gene polymorphism to Hardy-Weinberg law in the control group was tested with the chi-square test with 1 degree of freedom, without Yates correction, and the difference in the genotypes distribution in the control group and among the patients - with the chi-square test with 2 degrees of freedom. To assess the extent of the relative quality of statistical models was used Akaike information criterion (AIC) [29].

2.5 Statistical analysis

The statistical analysis was performed using applications MYSTAT 12 (Systat Software Inc., USA) and Scout 2008 Version 1.00.01 (USA Environmental Protection Agency, USA). The reliability of data for independent samples was calculated according to t-test Student (with the distribution of ranges close to normal), or U-criterion Wilcoxon-Mann-Whitney (with uneven distribution). The analysis of qualitative features was performed according to the χ^2 criterion. The difference was considered reliable at $p<0.05$.

3. Results and Discussion

Among the patients there were 23 (18.7%) women and 100 (81.3%) men. The patients' average age was 45.1±5.19 years for males, 53.2±7.07 years for females (from 23 to 77).
The possible inheritance models of edematous pancreatitis (dominant, recessive, over-dominant and additive) were analysed using logistic regression considering the analysed genes polymorphism (Table 1). In the absence of homozygotes for genes mutations *PRSS1 (365G>A), CFTR (delF508), SPINK1 (215G-A)* and *TNF-α (G-308A)* it became possible to count co-dominant, over-dominant and additive models for which criterion Akaike was: for gene *PRSS1* – 11.53, for gene *CFTR* – 10.90, for gene *TNF-α* – 10.24. The received data show that edematous pancreatitis (AP, ECP) is not inherited in the investigated population as the dominant feature, with a somewhat higher chance of its appearance in the presence of *GG*-genotype of genes *PRSS1, SPINK1* and *TNF-α*, *M*-allele of the gene *CFTR* ($p>0.05$).

Table 1: The inheritance dominant model of edematous pancreatitis taking into account the genes *PRSS1 (365G>A), CFTR (delF508), SPINK1 (215G-A)* and *TNF-α (G-308A)* polymorphism

Genotype	Control group, n (%)	Case, n (%)	OR [95% CI]	p	AIC
Depending on the polymorphic variants of gene PRSS1					
GG	38 (95.0%)	117 (95.1%)	1.0	0.98	11.53
GA + AA (0)	4 (5.0%)	6 (4.9%)	0.97 [0.21–6.84]		
Depending on the polymorphic variants of gene CFTR					
NN	39 (97.50)	98 (97.0)	1.0	0.88	10.90
NM + MM (0)	1 (2.50)	3 (3.0)	1.19 [0.15-24.54]		
Depending on the polymorphic variants of gene SPINK1					

GG	40 (100.0)	62 (98.41)	1.0	1.0	9.03
GA + AA (0)	0	1 (1.59)	-		
Depending on the polymorphic variants of gene TNF-α					
GG	27 (67.50)	9 (81.82)	1.0	0.36	10.24
GA + AA (0)	13 (32.50)	2 (18.18)	0.46 [0.06-2.12]		

Note: OR – Odds Ratio; 95% CI – confidence interval; AIC – Akaike information criterion

It was established, having analysed the inheritance models of AP and ECP based on *C-590T* polymorphism of gene *IL-4* (Table 2), that the recessive model was the most effective with AIC 16.03. This model shows that edematous pancreatitis in the general population is inherited as recessive sign that has higher chances to manifest in the presence of the *TT*-genotype in the promoter region of gene *IL-4* than the *C*-allele (OR=1.21; 95% CI: 0.34-5.66, $p>0.05$).

Table 2: Inheritance models of pancreatitis based on *C-590T* polymorphism of gene *IL-4*

Genotype	Control group, n (%)	Case, n (%)	OR [95% CI]	p	AIC
Dominant model					
CC	26 (65.0)	58 (57.4)	1.0	0.41	15.42
CT + TT	14 (35.0)	43 (42.6)	1.38 [0.65–3.0]		
Recessive model					
CC + CT	37 (92.50)	92 (91.10)	1.0	0.79	16.03
TT	3 (7.50)	9 (8.90)	1.21 [0.34–5.66]		
Over-dominant model					
CC + TT	29 (72.50)	67 (66.30)	1.0	0.48	15.6
CT	11 (27.50)	34 (33.70)	1.34 [0.61 – 3.09]		
Additive model					
CC	-	-	1.0	0.46	15.54
2 TT + CT	-	-	1.25 [0.71–2.31]		

Note: OR – Odds Ratio; 95% CI – confidence interval; AIC – Akaike information criterion.

We have analysed the risk of pancreatitis in the population taking into consideration genetic component with the help of Clinical Epidemiology methods (Tables 3-5). The polymorphism of gene *PRSS1 (365G>A)* is not a risk factor of AP, ECP, neither of alcohol nor of biliary origin.

The polymorphism of gene *CFTR (delF508)* also is not a risk factor of AP or ECP appearance (Table 3). The polymorphic variants of gene *CFTR (rs 113993960),* as risk factors of alcohol/biliary edematous pancreatitis, corresponded such of AP and ECP in the connection with the same genotype frequencies for corresponding distribution.

Table 3: Polymorphism of gene CFTR (rs 113993960) as a risk factor of edematous pancreatitis (acute/exacerbation of chronic)

Index	A potential risk factor of			
	acute pancreatitis		exacerbation of chronic pancreatitis	
	NN	NM	NN	NM
RelR	0.98	1.87	1.03	-
OR	0.52	1.92	-	-
95%CI RR	0.91-1.05	0.20-17.41	0.98-1.08	-
95%CI OR	0.05-5.19	0.19-19.10	-	-
p	>0.05	>0.05	>0.05	-

Note: RelR – relative risk; OR – Odds Ratio; 95% CI – confidence interval; 95%CI RR – confidence interval of Risk Ratio; 95%CI OR – confidence interval of Odds Ratio.

Polymorphic variants of gene *SPINK1 (215G-A)* are not risk factors of edematous pancreatitis: by the order of appearance - neither AP (RelR=0.97, 95%CI RR: 0.92-1.03, $p>0.05$) nor ECP ($p>0.05$), by the etiological factor - neither alcohol nor biliary ($p>0.05$).

Gene *TNF-α (G-308A)* polymorphisms also is not a risk factor of AP and ECP appearance, neither of biliary nor of alcoholic origin.

It was found that *TT*-genotype of gene *IL-4* increases the risk of ECP almost doubles it (OR=1.93; $p>0.05$), whereas the carrier of *C*-allele on the contrary is protective and makes the chances of exacerbation of chronic pancreatitis significantly lower in the investigated population (OR=0.41; 95%CI OR: 0.16-1.03; p=0.046) (Table 4). The analysed gene *IL-4* polymorphism is not associated with the risk of alcoholic/biliary edematous pancreatitis (Table 5).

Table 4: Gene *IL-4 (C-590T)* polymorphism as a risk factor of edematous pancreatitis (acute/exacerbation of chronic)

Genotypes of gene IL-4		RelR	OR	95%CI RR	95%CI OR	P
Acute pancreatitis	CC	1.01	1.03	0.76-1.35	0.45-2.36	>0.05
	TC	1.02	1.03	0.54-1.94	0.43-2.49	>0.05
	TT	0.83	0.82	0.20-3.53	0.17-3.88	>0.05
Exacerbation of chronic pancreatitis	CC	0.66	0.41	0.43-1.03	0.16-1.03	0.046
	TC	0.66	0.41	0.43-1.03	0.16-1.03	0.046
	TT	1.80	1.93	0.46-7.02	0.43-8.70	>0.05

Note: RelR – relative risk; OR – Odds Ratio; 95% CI – confidence interval; 95%CI RR – confidence interval of Risk Ratio; 95%CI OR – confidence interval of Odds Ratio.

Table 5: Gene *IL-4 (C-590T)* polymorphism as a risk factor of alcoholic/biliary edematous pancreatitis

Genotypes of gene IL-4		RelR	OR	95%CI RR	95%CI OR	p
Alcoholic pancreatitis	CC	0.94	0.84	0.69-1.27	0.37-1.91	>0.05
	TC	1.19	1.29	0.65-2.20	0.54-3.07	>0.05
	TT	0.78	0.77	0.18-3.33	0.16-3.63	>0.05
Biliary pancreatitis	CC	0.79	0.57	0.54-1.16	0.23-1.42	>0.05
	TC	1.28	1.43	0.66-2.49	0.54-3.76	>0.05
	TT	1.80	1.93	0.46-7.02	0.43-8.70	>0.05

Note: RelR – relative risk; OR – Odds Ratio; 95% CI – confidence interval; 95%CI RR – confidence interval of Risk Ratio; 95%CI OR – confidence interval of Odds Ratio.

Population and race analysis showed that the frequency of the minor *TT*-genotype of gene *IL-4* among the investigated by us people (7.50% - in the control group, 8.91% - research) is somewhat higher, than on the average for the aggregate europeoid populations (0-2% - in the general population, 5-38% - among the patients with pancreatitis, $p>0.05$), significantly lower than among the equatorial and the overwhelming majority of Asian race people (P_{TT}=0.54-0.62, versus P_{TT} =0.075-0.089; $p<0.05$). The frequency of wild *C*-allele in our studies is substantially higher than such for the equatorial race and some populations of Asian race (R_C=0.21-0.29 versus R_C=0.74-0.79, $p<0.05$) and did not differ from that for europeoids (R_C=0.83-0.86; $p>0.05$), respectively (Population Diversity … rs2243250).

The data as to population and racial differences of the frequencies of delF508 polymorphism of gene CFTR are unavailable for comparison at the official website of NCBI [30]. However, are available the data of individual researchers as to F508del mutation of gene CFTR: in families with lung cystic fibrosis this mutation occurs with a frequency of 46 to 67.1% in the Spanish-Americans and Latyno-Americans [31, 32], 18.51% - in Kermanshah Province, Western Iran [33], whereas in the English with pancreas cystic fibrosis (associated with the Alcohol intake) significantly less - from 5.3 to 13.4% of cases [34], in the patients with disseminated pulmonary bronchiectasis of unknown origin, as well as with COPD - in 56% of cases [35, 36], for idiopathic CP - in 37% of cases [37], in the US population (ethnic group Schmiedeleut (S-leut) Hutterites – of Ukrainian origin, n=1482) were found no homozygotes of Phe508del mutation of gene CFTR in general and only 32 heterozygotes, which amounted to 0.022 of frequency (2.2%) [6]. In our study, the mutation of the gene in the heterozygous state occurred in 4.69% of cases (these were people with alcohol AP origin, cystic changes of pancreas parenchyma), and in 2.50% of cases - in the control group.

The population differences in frequencies 215G-A of the polymorphism of gene *SPINK1* (Gene ID 6690) at the time of the research the data are available only for individual researchers and are not available for the comparison on the official website of NCBI (http://www.ncbi.nlm.nih.gov/SNP/snp). In some European populations this mutation occurs with the following frequency: in patients with idiopathic chronic pancreatitis - in 2.61-11.30% of cases, 9.1% according to Masson *et al*. [3], in the control (total population) - in 1-1.67% [9, 26] which did not differ significantly from our results (available mutation in 2.78% of patients with edematous pancreatitis, the absent in the control). In the Asians this gene *SPINK1* mutation occurs with the frequency 3.7-4.4% of patients with pancreatitis without diabetes, whereas as to fibro-calculous pancreatic diabetes - at the rate of 33% [13], and mutations in the general population was not found at all [17]. Instead, there are few data where this mutation among the europeoid race occurs in patients with acute and chronic edematous pancreatitis more likely in 22.92-54.55% of cases with advantage of the necrotic form of AP [38, 39], but the number of observations in these studies was to 100 people, and sample was characterized by high heterogeneity.

The data as to population and racial differences of frequencies 365G>A of the polymorphism of gene *PRSS1* on NCBI site are limited [40] and are related to the aggregate populations: the incidence frequency of *G*-allele - 99.99%, *A*-allele – 0.01%, respectively. In our studies - 96.57% and 3.42%, respectively, and that was not significantly different from the above mentioned results and according to other studies [41, 42]. However, the study of the gene mutation rate PRSS1 (rs111033565) in the Polish population [8] with alcohol, or idiopathic chronic pancreatitis was 33% and 21.4%, and in the control - 4.3%; 9.1% in young French patients [3]; according to the European Registry of Hereditary Pancreatitis and Pancreatic Cancer a high level of the mutations is observed in the families with *PRSS1*-dependent hereditary pancreatitis - on average in 52% of the investigated families from 14 European countries [14].

As to the racial and population differences *G-308A* of the polymorphism of gene *TNF-α*, the frequency of wild *G*-allele of gene *TNF-α* among the residents of Bukovina (P_G=0.84-0.91) and also of the minor *A*-allele (R_A=0.09-0.16) correspond to such on average of europeoid populations (P_G=0.78-0.95 and R_A=0.05-0.22, $p>0.05$), indicating a rather high population homogeneity according to polymorphic locus. However, there are significant differences when compared with some Asian race populations in which the frequency of allele has significant differences, indicating high heterogeneity (P_G=0.22-0.89 and R_A=0.02-0.98). The frequency of the *A*-allele in our study is slightly lower than such in the population of sub-saharaoid Africa residents (P_G=0.91-0.94 versus R_A=0.06-0.09; $p<0.05$).

4. Conclusion

The analysis of inheritance models of AP and ECP taking into consideration the polymorphic variants of gene *IL-4* (rs 2243250) found that the most effective is the recessive model with the Akaike information criterion 16.03. This model indicates that edematous pancreatitis in the general population is inherited as a recessive feature, which has higher chances to manifest at the presence of the *TT*-genotype in the promoter region of gene *IL-4*, than the *C*-allele (OR=1.21; 95% CI: 0.34-5.66, $p>0.05$).

The genes *PRSS1 (365G>A), CFTR (delF508), SPINK1 (215G-A)* and *TNF-α (G-308A)* polymorphism is not the risk factor of the appearance of AP, ECP neither of alcoholic nor of biliary origin. *TT*-genotype of gene *IL-4* increases the risk ECP almost doubles it (OR=1.93; $p>0.05$), whereas the C-allele carrier is contrary protective and makes the chances of

exacerbation of chronic pancreatitis credibly lowest in the investigated population (OR=0.41, 95%CI OR: 0.16-1.03; p=0.046). The analysed gene IL-4 polymorphism is not associated with the risk of neither alcohol nor biliary edematous pancreatitis.

In perspective we plan to analyse the association of analysed genes' haplotypes from the position of edematous pancreatitis development risk.

5. Limitations of the Study: The study was limited by the number of enrolled patients.

6. Conflict of Interest: None declared.

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