



## Polymorphism of matrix metalloproteinase-2 (C<sup>-1306</sup>→T) and tissue inhibitors of metalloproteinase-2 (G<sup>303</sup>→A) genes in patients with enterocutaneous fistula

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**ABSTRACT**

*Aim:* To analyze the frequency of polymorphic variants of matrix metalloproteinase-2(C<sup>-1306</sup>→ T) and tissue inhibitors of metalloproteinase-2(G<sup>303</sup>→ A) genes in patient's with enterocutaneous fistula. *Materials and methods:* The object of the study comprises 63 patients with enterocutaneous fistula and connective tissue pathology who were treated in the Shalimov National Institute of Surgery and Transplantology during 2016-2019. Laboratory, genetic, histological studies and statistical analysis were performed. *Results:* As a result of genetic and statistical analysis of the matrix metalloproteinase-2(C<sup>-1306</sup>→T) and tissue inhibitors of metalloproteinase-2 (G<sup>303</sup>→A) gene single nucleotide polymorphisms, genotype variants have been identified that are associated with the risk of enterocutaneous fistula development. All models of inheritance were analyzed and the best model with the lowest Akaike information criterion, which turned out to be a recessive model, has been determined. *Conclusions:* Enterocutaneous fistula is 1,58 times more common in carriers of homozygous GG genotype of the tissue inhibitors of metalloproteinase-2(G<sup>303</sup>→A) gene and twice less common in heterozygotes GA (21.1% vs. 40%, p=0.057). Carriers of minor homozygotes of AA genotype in the group with enterocutaneous fistula were not detected, while a similar genotype in the control group was found in 10% of cases. It's statistically significant that in the group of patients with enterocutaneous fistula the single nucleotide polymorphisms of the matrix metalloproteinase-2(C<sup>-1306</sup>→T) gene's promoter doesn't differ from the control group.

**Keywords:** Enterocutaneous fistula, matrix metalloproteinase-2, tissue inhibitors of metalloproteinase-2, gene polymorphism.

**1. INTRODUCTION**

Enterocutaneous fistula (ECF) is serious complication in abdominal surgery and is a real threat to the life of the patient. So far, there is no single point of view in the surgical community regarding the causes of intestinal fistulas development and surgical tactics in the development of these complications. The incidence of the ECF is 1-2% of all abdominal operations, but they create many problems both from the surgical point of view and treatment of the patient (Schechter et al., 2009). A simple and convenient classification based on anatomical, functional (flow rate in ml/day) and etiological characteristics of the ECF (Teixeira et al., 2009) is often found in English sources. An organ of origin is another classification used for ECF and is useful as well in the consideration of management options: type I (abdominal, esophageal, gastroduodenal), type II (small bowel), type III (large bowel), and type IV (enteroatmospheric, regardless of origin) (Schein et al., 1991).

Postoperative ECF account for 75-85% of all intestinal fistulas. Postoperative complications in the form of fistula often develop after oncological surgeries, surgeries for inflammatory bowel disease, and acute intestinal obstruction (Rene et al., 2001). According to the literature, small bowel fistulas open into the abdominal cavity in 29-32%, through the abscess cavity in 24.3% of cases, through the eventrated wound - 9.3% (Whelan and Ivatury, 2011). Mortality in the development of ECF in the early postoperative period is 16.5-57.5%, in the acute period (unformed intestinal fistulas) - 20.0-80.0%, with high ECF- 82-90% (Williams et al., 2010). The main causes of death are progressive peritonitis, sepsis, intoxication, malnutrition, fluid and electrolyte abnormalities, hepatic and renal failure, intestinal insufficiency (Lloyd et al., 2006). Despite improvements in nutritional and metabolic support, antimicrobial therapy, improved wound care, improved surgical techniques, the mortality rate remains extremely high (Whelan and Ivatury, 2011). There are almost no publications about the role of undifferentiated dysplasia of the connective tissue (UDCT) in the development of intestinal fistulas.

Given the almost unexplored role of genetic predisposition in the development of postoperative complications, namely the ECF, we set a goal to study the polymorphism of genes encoding matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of matrix metalloproteinase-2 (TIMP-2). The choice of these genes was not accidental - we were guided by the main known pathophysiological mechanisms involved in the formation of the intestinal anastomosis (Lloyd et al., 2006), intestinal healing processes after iatrogenic injury (Berry et al., 1996), intestinal fistula formation in inflammatory bowel diseases (van Haaften et al., 2017). Matrix metalloproteinases (MMPs) are a group of enzymes represented by cysteine, serine, aspartyl, and metal-dependent proteinases. They belong to Zn<sup>2+</sup> - and Ca<sup>2+</sup>-dependent endopeptidases, which are involved in the remodeling of connective tissue due to the destruction of its organic components at normal pH values. MMPs play a major role in the metabolism of connective

tissue proteins. These enzymes are also involved in many physiological and pathological processes. They are also able to model the activity of growth factors, cytokines, and their receptors. MMP-2,9 show a high affinity for type IV collagen, so they are sometimes called type IV collagenases. MMP-2 occupy a central position in the regulating of the balance between the processes of synthesis and proteolysis in the extracellular matrix, affect the implementation of physiological processes and pathological changes in the body (Visse et al., 2003).

The main regulators of matrix metalloproteinases are tissue inhibitors of metalloproteinases - TIMPs . All 4 groups of TIMPs can inhibit the proteolysis of latent forms of MMP and inhibit the active forms of MMP, but TIMP-1 is more active against MMP-9, and TIMP-2 shows specificity for MMP-2 (Fassina et al., 2000). During the analysis of the literature, we found publications on the study of MMPs expressions and their role on the remodeling of extracellular matrix in the anastomotic leak (Stumpf et al., 2009; Agren et al., 2006), inflammatory bowel diseases (Martin et al., 2007; M. Scharl et al., 2016). There are reports of an association between the allelic polymorphism of the HSP70-2 heat shock gene and the development of external ECF (Jun Chen et al., 2014). However, we have not found publications on the study of genetic polymorphism of matrix metalloproteinases and their regulators in terms of the development of ECF.

### Aim

To analyze the frequency of polymorphic variants of genes MMP-2 (C<sup>-1306</sup>→ T) and TIMP-2 (G<sup>303</sup>→A) in patients with ECF.

## 2. MATERIALS AND METHODS

The object of the study comprises 63 patients, all treated at the Shalimov National Institute of Surgery and Transplantology during 2016-2019, 19 of 63 patients suffered external ECF (experimental group 2), 44 of 63 patients had phenotypic signs of UDCT (experimental group 1). For the assessment of genetic polymorphism in the population, 80 practically healthy people have been examined (control group), who were matched by gender and age with experimental groups. For the assessment of connective tissue, we analyzed free hydroxyproline in the serum and urinary glycosaminoglycans. UDCT has been diagnosed with a proven technique (Ukrainian patent for utility model №120158 UA). The stage of dysplasia was evaluated using the original clinical screening scale, which was based on the table of the severity criteria of connective tissue dysplasia made by T.Y. Smolnova (2003) (Usenko and Voitiv, 2017).

Genetic studies were performed in the laboratory of the Department of General and Molecular Pathophysiology at the Bogomoletz Institute of Physiology NAS of Ukraine. The collection of the buccal epithelium was performed using buccal brushes with the upcoming freezing of the samples at the temperature of -20°C. DNA for the genotyping was extracted from the samples using Diatom™ Prep 200 (Isogen Laboratory, RF) following the manufacturer's protocol.

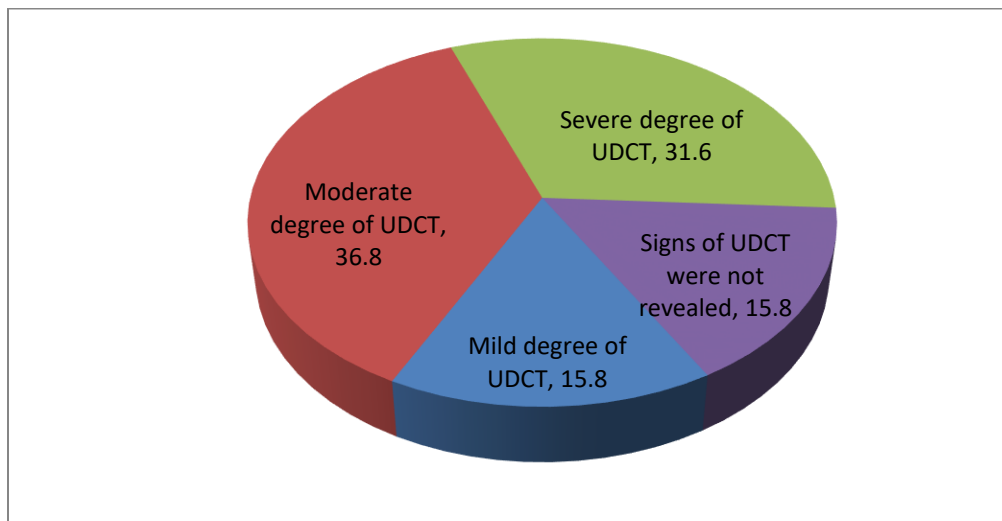
The following polymorphisms were studied by real-time PCR: C<sup>-1306</sup> → T (MMP2), rs243865 and G<sup>303</sup> → A (TIMP2), rs9900972. Amplification reactions were performed using the Fast Real-time PCR System (Applied Biosystems, USA) in a final reaction volume of 20 µl containing 2X TaqMan Universal Master Mix (Applied Biosystems, USA), assay C\_1792560\_10 and template DNA. Amplification of gene fragments consisted of a denaturation step at 95° C for 20 s, followed by 40 cycles of amplification at 95° C for 3 s and 60° C for 30 sec. Data analysis was performed with 7500 Fast Real-Time PCR Software (Applied Biosystems, Foster City, USA).

The main part of the statistical analysis was performed using the program "Statistica 7.0" (SPSS) and Excel 2000. Nominal data were presented in the form of quantitative and percentage values. The significance of differences in mean values in groups with different genotypes was determined using the method of one-way analysis of variance (URL: <http://www.dgmp.kyiv.ua/index.php/snip-ka>). The correspondence of genotype distribution was checked using the Hardy-Weinberg test. Pearson's  $\chi^2$  test was used to compare the distribution of genotypes in the experimental and control groups.

## 3. RESULTS

In the experimental group of patients with ECF, the vast majority were patients after emergency surgery for acute intestinal obstruction, disseminated peritonitis, destructive pancreatitis, acute destructive appendicitis. In 42.2% (8 of 19) cases, the cause of fistula development was the anastomotic leak (6) and mechanical damage to the bowel wall during viscerolysis (2). In 2 cases, fistulas occurred in patients with giant recurrent ventral hernias (strangulation of the intestine in the hernia sac - 1, fixation of the intestine to the mesh graft - 1); 2 colonic fistulas in patients with destructive pancreatitis (bedsores from drainage - 1, peripancreatic abscess - 1); damage to the duodenum during right nephrectomy - 1, perforation of the diverticulum of the colon - 2, perforation of the cecum and ascending colon due to abscesses of the peritoneal cavity. In the remaining cases (2) no convincing reasons were identified.

The criterion for exclusion from the experimental group were fistulas in patients with ulcerative colitis, Crohn's disease, and ECF that developed after radiation therapy. In examined patients with anastomotic leak in hollow digestive organs, signs of UDCT were found in 13 (76.47%) patients. The following phenotypic pathologies of UDCT were most commonly encountered: visceral pathology (84.2%), vascular pathology (73.7%), arrhythmias (63.1%). The study of phenotypic signs of UDCT in the group of patients with external ECF showed that 3 patients (15.8%) - had a mild UDCT, 7 patients (36.8%) - had moderate, and 6 patients (31.6%) had a severe degree of UDCT. In 3 patients (15.8%), signs of the pathology of the connective tissue were not detected (Diagram 1).



**Diagram 1** Severity of UDCT in patients with ECF (%)

The level of serum hydroxyproline in the group of patients without phenotypic signs of connective tissue dysplasia was  $37.4 \pm 4.7$   $\mu\text{mol/L}$ , which is 76% higher than the control group ( $21.2 \pm 0.8$   $\mu\text{mol/L}$ ). Such changes are apparently due to increased proteolytic activity in patients with ECF and the anastomotic leak. This confirms the data of some authors that ECF development leads to a pronounced and persistent mismatch in the proteinase system - inhibitors of blood proteinases. It is the hyperactivation of proteolytic systems of the body against the background of reduction of inhibitory potential that is regarded as one of the key pathogenetic links of endogenous intoxication.

**Table 1** The distribution of polymorphic variants of genes MMP-2 ( $C^{-1306} \rightarrow T$ ), rs243865 and TIMP-2 ( $G^{303} \rightarrow A$ ), rs9900972 in the studied groups

The studied gene		Control group n=80 (%)	Experimental group 1 (with phenotypic signs of UDCT) n=44 (%)	Experimental group 2 (with ECF) n=19 (%)
MMP-2 ( $C^{-1306} \rightarrow T$ )	CC	38 (47.5%)	26 (59.1%)	10 (52.6%)
	CT	34 (42.5%)	16 (36.4%)	7 (36.8%)
	TT	8 (10%)	2 (4.5%)	2 (10.5%)
Hardy-Weinberg test ( $\chi^2, p$ )		$\chi^2=0.01, p>0.05$	$\chi^2=0.05, p>0.05$	$\chi^2=0.21, p>0.05$
Тест $\chi^2, (\chi^2, p)$		-	$\chi^2=2.051, p>0.05$	$\chi^2=0.206, p>0.05$
TIMP-2 ( $G^{303} \rightarrow A$ ),	GG	50 (50%)	24 (54.5%)	15 (78.9%)
	GA	32 (40%)	15 (34.1%)	4 (21.1%)
	AA	8 (10%)	5 (11.4%)	0 (%)
Hardy-Weinberg test ( $\chi^2, p$ )		$\chi^2=0.18, p>0.05$	$\chi^2=1.15, p>0.05$	$\chi^2=0.26, p>0.05$
Тест $\chi^2, (\chi^2, p)$		-	$\chi^2=0.425, p>0.05$	$\chi^2=5.73, p=0.057$

When studying the dynamics of changes in serum hydroxyproline levels, it was found that an increase in the collagenolytic activity of glycosaminoglycans and free hydroxyproline levels had a direct correlation with the severity of UDCT. With a mild degree of UDCT, the level of serum hydroxyproline was  $(48.2 \pm 2.6)$   $\mu\text{mol/L}$ , moderate  $(75.1 \pm 3.6)$   $\mu\text{mol/L}$  and severe  $(114.3 \pm 3.9)$   $\mu\text{mol/L}$ , which is 5.5 times higher than in the control group and 3 times higher than in patients with ECF without clinical signs of connective tissue

dysplasia. To identify the possible association of single nucleotide polymorphisms (SNP) variants of the MMP-2 (C<sup>-1306</sup> → T) and TIMP-2 (G<sup>303</sup> → A) genes with the risk of ECF, we performed a one-way analysis of variance of the frequency of genotypes in the studied groups of patients (table 1).

In the analysis of models of inheritance of the MMP-2 gene (C<sup>-1306</sup> → T), namely codominant, dominant, recessive, supradominant and additive in the control group (n=80) and the experimental group 1 with phenotypic signs of UDCT (n=44), it was found that the distribution of genotypes corresponds to the Hardy-Weinberg law (p>0.05). Using the  $\chi^2$  test with 2 degrees of freedom, we were not able to detect statistically significant differences in the distribution of genotypes in the group of sick people and the group of practically healthy people (p> 0.05). Having analyzed all inheritance models, we selected the best model with the lowest Akaike Information Criterion (AIC). Such a model turned out to be a recessive model, for which the table shows the values of the odds ratio, statistical significance, as well as the AIC (Table 2).

**Table 2** The odds ratio for a recessive model inheritance in patients with phenotypic signs of UDCT. Odds ratio with 95% confidence interval.

Genotype	Control group n=80 (%)	Experimental group 1 (with phenotypical signs of UDCT) n=44 (%)	Odds ratio	p-value	AIC
CC+CT	72 (90%)	42 (95.5%)	1,00		
TT	8 (10%)	2 (4.5%)	0.43 (0.06 – 1.81)	0.3	16.12

Analysis of the multiplicative model of inheritance of the MMP-2 gene (C<sup>-1306</sup> → T), comparing the control group (n=80) and experimental group 2 with external ECF(n=19) showed compliance with the distribution of genotypes to Hardy-Weinberg's law (p>0.05), which was tested in the control group using the test  $\chi^2$  with 1 degree of freedom, without Yates correction. Using the test  $\chi^2$  with 2 degrees of freedom, we did not find statistically significant differences in the distribution of genotypes in the group of sick people and the group of practically healthy people (p>0.05). After analyzing all models of inheritance, we chose the best model with the lowest AIC (Table 3).

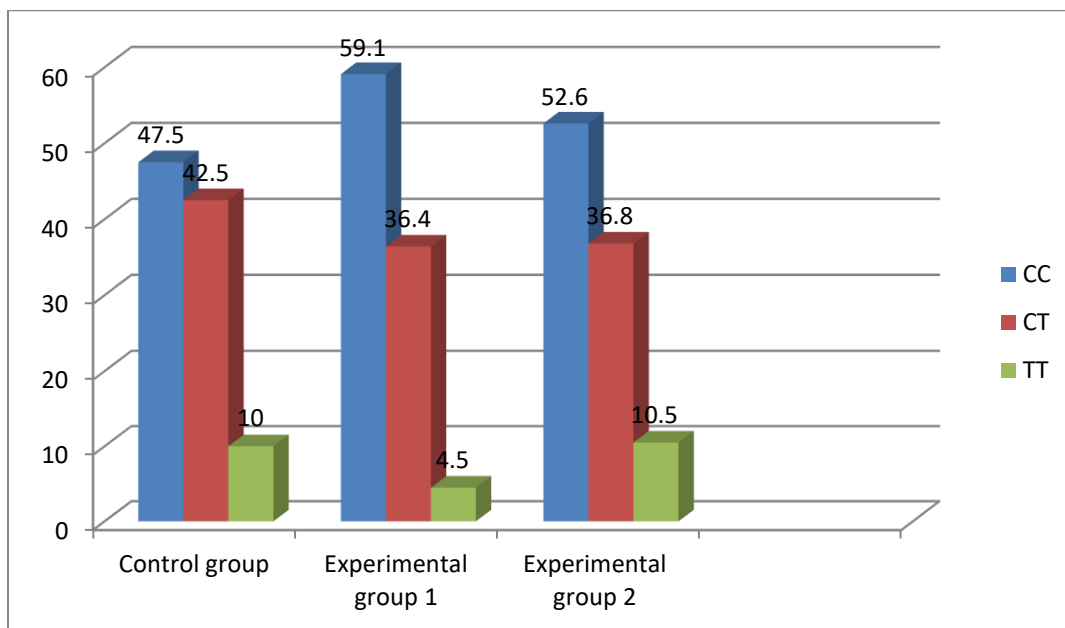
**Table 3** The odds ratio for a recessive model inheritance in patients with with ECF. Odds ratio with 95% confidence interval интервалом

Genotype	Control group n=80 (%)	Experimental group 2 (with ECF) n=19 (%)	Odds ratio	p-value	AIC
CC+CT	72 (90%)	17 (89.5%)	1.00		
TT	8 (10%)	2 (10.5%)	1.06 (0.15 – 4.71)	0.95	14.14

With the analysis of the frequency of MMP-2 genes allelic polymorphism, it was detected that carriers of dominant in all groups of CC genotype were the most in the group with phenotypic signs of UDCT (experimental group 1): 59.1% versus 47.5% (p> 0.05) in the control group. While carriers of homozygous TT-genotype, in the experimental group 1, was more than twice less (4.5% vs 10% (p> 0.05)). It is noteworthy that in the experimental group 2 with intestinal fistulas, the frequency distribution of the MMP-2 gene promoter polymorphism, in general, corresponded to the indicators of the control group on the CC, CT and TT variants (Diagram 2).

In the analysis of TIMP-2 inheritance models (G<sup>303</sup> → A), in the control group (n=80) and experimental group 1 with phenotypic signs of connective tissue pathology (n=44), we could not find statistically significant differences in the distribution of genotypes in the group of patients and the group of almost healthy people (p>0,05). The conformity of the genotype distribution to Hardy-Weinberg's law in the control group was checked using the  $\chi^2$  test with 1 degree of freedom, without the use of Yates correction (table 4).

In the analysis of TIMP-2 inheritance models (G<sup>303</sup>→A), in the control group (n=80) and experimental group 2 with external ECF (n=19), differences in the frequency of genotype distribution were on the verge of statistical significance: p=0.057. The conformity of the genotype distribution to Hardy-Weinberg's law in the experimental group 2 was checked using the  $\chi^2$  test with 1 degree of freedom, without the use of Yates correction. After analyzing all models of inheritance, we chose the best model with the lowest AIC (Table 5).



**Diagram 2** Frequency distribution of allelic polymorphism (%) of the promoter MMP2(C<sup>-1306</sup> → T) gene

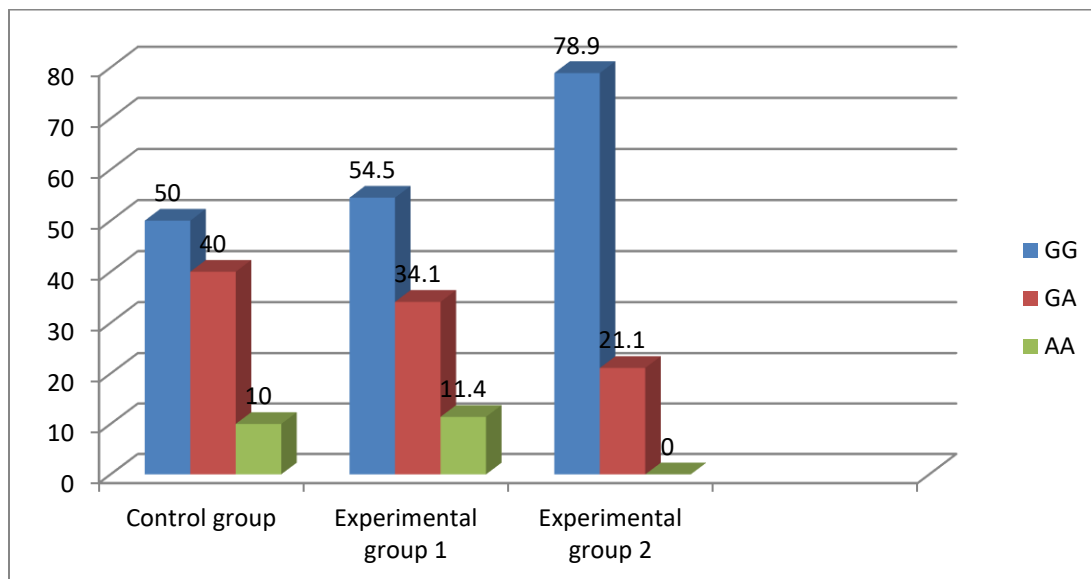
**Table 4** The odds ratio for a recessive model inheritance in patients with phenotypic signs of UDCT. Odds ratio with 95% confidence interval.

Genotype	Control group n=80 (%)	Experimental group1 (with phenotypic signs of UDCT) n=44 (%)	Odds ratio	p-value	AIC
GG+GA	72 (90%)	39 (88.6%)	1.00		
AA	8 (10%)	5 (11.4%)	1.15 (0.33 - 3.7)	0.81	16.1

**Table 5** The odds ratio for a recessive model inheritance in patient's with ECF. Odds ratio with 95% confidence interval

Genotype	Control group n=80 (%)	Experimental group 2 (with ECF) n=19 (%)	Odds ratio	p-value	AIC
GG+GA	72 (90%)	19 (100%)	1.00		
AA	8 (10%)	0 (0%)	0 (NA - 1.96545419474352e+266)	1	15.07

In the examined population in the control group and experimental group 1, the distribution of carriers of GG, GA and AA genotypes was significantly similar. However, in the group of patients with external ECF (experimental group 2), the distribution of genotypes carriers was significantly different. Thus, the dominant homozygous GG variant 1.58 times exceeded the values of the control group ( $p=0.057$ ) and 1.46 times exceeded the values of experimental group 1 ( $p>0.05$ ). Heterozygous GA genotype in the experimental group 2 was twice less common than in the control group (21.1% vs. 40%,  $p=0.057$ ) and 1,6 times less common than in experimental group 1 ( $p>0.05$ ). Carriers of homozygous AA genotype in the group with ECF were not detected, while a similar variant in control and experimental group 1 was found in 10% and 11.4% of cases (Diagram 3).



**Diagram 3** Frequency distribution of allelic polymorphism (%) of the promoter of the TIMP2 ( $G^{303} \rightarrow A$ ) gene

#### 4. DISCUSSION

Our data on the study of polymorphic variants of the MMP-2 ( $C^{-1306} \rightarrow T$ ) and TIMP-2 ( $G^{303} \rightarrow A$ ) genes in the Ukrainian population ( $n=80$ ) generally correspond to populations of Europe and the USA (<https://www.ncbi.nlm.nih.gov/snp/rs243865>; <https://www.ncbi.nlm.nih.gov/snp/rs9900972>). The closest genotypic variations in the studied genes were populations of Austria (Mossbock et al., 2010) and the Netherlands (van Diemen et al., 2011). Moreover, we found significant differences when compared with the African and Asian populations (Xu et al., 2004). Interestingly, in these populations, the frequency of the main C allele of the MMP-2 gene (rs243865) was 93.7% (Africa) and 90% (Asia), which significantly exceeds the indices of our control group (76%) and the European population (75.5%). Whereas, the minor T allele was found in 24% of the control group, and 10% (Asia) and 6.7% (Africa), respectively (Li et al., 2010).

As a result of genetic and statistical analysis of the polymorphism of the MMP-2 ( $C^{-1306} \rightarrow T$ ) and TIMP-2 ( $G^{303} \rightarrow A$ ) genes, variants of genotypes associated with the risk of development of external ECF were determined. When analyzing the frequency of allelic polymorphism of the MMP-2 gene in the experimental group 2, no statistically significant differences were found compared with the control group. The frequency distribution of the polymorphism of the MMP-2 gene promoter, in general, corresponded to the parameters of the control group for CC, CT, and TT variants. At the same time, differences in the frequency of genotype distribution of the TIMP-2 gene ( $G^{303} \rightarrow A$ ), in the control group and experimental group 2 with external ECF were on the verge of statistical significance:  $p=0.057$ . Thus, the dominant homozygous GG variant was 1.58 times higher than the control values ( $p=0.057$ ) and 1.46 times higher than the experimental group 1 values ( $p>0.05$ ). Heterozygous GA genotype in experimental group 2 was twice less common than in the control group (21.1% vs. 40%,  $p=0.057$ ) and 1.6 times less common than in experimental group 1 ( $p>0.05$ ). Carriers of homozygous AA genotype in the group with ECF were not detected, while a similar variant in the control and experimental group 1 was found in 10% and 11.4% of cases.

Given the role of matrix metalloproteinases and their inhibitors in the processes of synthesis and proteolysis, extracellular matrix remodeling, connective tissue protein metabolism, the ability to affect vascular permeability and angiogenesis, the relevance of their study in the context of the pathogenesis of external ECF development is undoubted. The correlation between the level of biochemical markers of collagen biodegradation and the severity of UDCT revealed, which is diagnosed on the basis of phenotypic, visceral manifestations, and instrumental examinations. This could serve as an informative diagnostic criterion of UDCT and could be used to predict the development and course of complications in patients with ECF. Such changes are apparently due to increased proteolytic activity in patients with ECF. This confirms the data of some authors that the anastomotic leak, intestinal fistulas, and development of peritonitis lead to a pronounced and persistent mismatch in the proteinase system - inhibitors of blood proteinases. It is the hyperactivation of proteolytic systems of the body against the background of the reduction of inhibitory potential that is regarded as one of the key pathogenetic links of endogenous intoxication.

In our view, the focus of future research on the pathogenetic factors of abdominal postoperative complications should be shifted to a more cellular and molecular level. Thus, a better understanding of the mechanisms of the intestinal fistula formation will contribute to the development of new diagnostic, prognostic, and therapeutic techniques. The differences we have identified in

allelic variants of MMP-2 (C<sup>-1306</sup>→T), and TIMP-2 (G<sup>303</sup>→A) genes in the groups with external ECF and connective tissue dysplasia are the basis for further study and research for molecular genetic markers that encode the main links in the pathogenesis of abdominal postoperative complications.

## 5. CONCLUSION

External ECF are 1.58 times more common in carriers of homozygous GG genotype of the TIMP-2 (G<sup>303</sup>→A) gene, and twice less common in heterozygotes of GA (21.1% vs. 40% p=0.057). Carriers of minor homozygotes of AA genotype in the group with ECF were not detected, while a similar genotype in the control group and experimental group 1 was found in 10% and 11.4% of cases. It's statistically significant that in the group of patients with external ECF the SNPs of the MMP-2 (C<sup>-1306</sup>→T) gene's promoter doesn't differ from the control group. Molecular genetic research can be a new promising area for the development of modern personalized diagnostic criteria and models for predicting the development and course of postoperative abdominal complications, including ECF. The presence of connective tissue dysplasia in patients with external ECF is an aggravating comorbid factor, which must be considered when choosing adequate surgical tactics and complex pathogenetically substantiated treatment.

### Abbreviation

AIC-	Akaike information criterion
ECF -	enterocutaneous fistula
MMP-2 -	matrix metalloproteinase-2
MMPs-	matrix metalloproteinases
SNP -	single nucleotide polymorphisms
TIMP-2 -	tissue inhibitor of matrix metalloproteinase-2
UDCT-	undifferentiated dysplasia of the connective tissue

### Declarations

The research highlighted in the article became a fragment of the research work of the Department of Surgery and Transplantology of the Shupyk National Medical Academy of Postgraduate Education on the topic: "Undifferentiated connective tissue dysplasia as a risk factor in abdominal surgery" State Registration Number 0118U001239, deadline 2018-2022.

### Ethics approval and consent to participate

This cohort study was conducted with the Ethics Committee of the Shupyk National Medical Academy of Postgraduate Education and is compliant with the terms of the Helsinki Declaration (prot. N4, 02.04.2018). All participants completed a written consent.

### Competing interests

The authors declare that they have no competing interest

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### Authors' contributions

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All authors read and approved the final manuscript.

- A - Work concept and design
- B - Data collection and analysis
- C - Responsibility for statistical analysis
- D - Writing the article
- E - Critical review
- F - Final approval of the article



### Data and materials availability

All data associated with this study are present in the paper.

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