Abstract. The article reveals the relationship between the development of external enterocutaneous fistulas and variants of the genotype distribution of MMP-2 (C-1306 → T) and TIMP-2 (G303 → A) genes. Based on the received data personalized way of forecasting of the enterocutaneous fistulas is developed. The object of the study were 19 patients with enterocutaneous fistulas of anastomoses, who were treated at the State Institution "NIST named after O.O. Shalimov ". Laboratory, genetic and statistical studies were conducted. When analyzing the frequency of allelic polymorphism of the MMP-2 gene, it was found that in the experimental group with enterocutaneous fistulas, the frequency distribution of the polymorphism of the MMP-2 gene promoter, in general, corresponding to the control group for SS, CT, and TT variants. In the analysis of TIMP-2 inheritance models (G303 → A), in the control groups (n = 80) and the experimental group (n = 19) we were able to find statistically significant differences in the distribution of genotypes (p <0.05). Thus, the dominant homozygous GG variant was 1.58 times higher than the control values (p = 0.057). Heterozygous GA genotype in the experimental group was twice less common than in the control (21.1% vs. 40%). Carriers of homozygous AA genotype in the group with enterocutaneous fistulas were not detected, while a similar variant in the control occurred in 10% of cases.

Keywords. enterocutaneous fistulas, genes MMP-2, TIMP-2, method of prediction.

Introduction. Enterocutaneous fistula is a serious complication in abdominal surgery that poses a real threat to the patient's life. To date, surgeons do not have a
single point of view on the causes of enterocutaneous fistula, treatment tactics in the development of these complications.

The incidence of enterocutaneous fistula is 1-2% of all abdominal surgeries [1]. In the literature, a simple and convenient classification based on anatomical, functional (flow rate in ml/day) and etiological characteristics of enterocutaneous fistula is most common [2]. There is a classification based on the etiology of fistula: type I (esophageal, gastroduodenal), type II (small intestine), type III (colon), and type IV (external fistula regardless of origin) [3].

Postoperative enterocutaneous fistulas make up 75-85% of all enterocutaneous fistulas. The postoperative complication in the form of fistula often develops after cancer surgery, surgery for inflammatory bowel disease, and acute intestinal obstruction [4-6].

Given the almost unexplored role of genetic predisposition in the development of postoperative complications, namely, enterocutaneous fistulas, we set out to study the polymorphism of genes encoding matrix metalloproteinase type 2 (MMP-2) and tissue inhibitor of matrix metalloproteinases 2 (TIMP-2) in this group of patients.

**Aim.** Develop a personalized method for predicting enterocutaneous fistulas based on polymorphism analysis of MMP-2 (C-1306 → T) and TIMP-2 (G303 → A) genes.

**Material and methods.** The object of the prospective study were 19 patients with enterocutaneous fistulas, who were treated at the State Institution "NIST named after O.O. Shalimov ". To assess the polymorphism of genes in the population surveyed 80 healthy people who are comparable in age and sex with the subjects. Genetic research was conducted in the laboratory of the Department of General and Molecular Pathophysiology of the Institute of Physiology O.O. Bogomolets of the National Academy of Sciences of Ukraine. Collection of the buccal epithelium was performed using buccal brushes, followed by freezing of samples and their storage at a temperature of -20 °C. The following polymorphisms were investigated by real-time PCR: C-1306 → T (MMP2), rs243865, and G303 → A (TIMP2), rs9900972.

The main part of the statistical analysis was performed using the program "Statistica 7.0" and Excel 2000. Nominal data are 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-factor statistical analysis (URL: http://www.dgmp.kyiv.ua/index.php/snip-ka). The conformity of the 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.

**Results.** To identify a possible association of polymorphic variants of the MMP-2 (C 1306 → T) and TIMP2 (G303 → A) genes with the risk of enterocutaneous fistula, we performed a one-way statistical analysis of the frequency of genotypes in the studied patient groups.

Analysis of the multiplicative model of inheritance of the MMP-2 gene (C-1306 → T), comparing the control group (n = 80) and the experimental group with enterocutaneous fistulas (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 the use of Yates correction. Using the $\chi^2$ test with 2 degrees of freedom, we did not find statistically significant differences in the distribution of genotypes in the group of patients and in the group of practically healthy people ($p > 0.05$).

After analyzing all models of inheritance, we chose the best model with the lowest information criterion Akaike.

When analyzing the frequency of allelic polymorphism of the MMP-2 gene, it was found that in the experimental group with enterocutaneous fistulas, the frequency distribution of the polymorphism of the MMP-2 gene promoter, in general, corresponding to the control group for SS, CT, and TT variants.

In the analysis of TIMP-2 inheritance models (G303 → A), in the control groups (n = 80) and the experimental group (n = 19) we were able to find statistically significant differences in the distribution of genotypes ($p < 0.05$).

In the analysis of allelic polymorphism of the TIMP-2 gene promoter (G303 → A), in the control groups (n = 80) and the experimental group (n = 19) with external enterocutaneous fistulas (n = 19) we were able to find significant differences that were on the verge statistical significance: $p = 0.057$ (table 1).
Table 1

Distribution of polymorphic variants of genes MMP-2 (C-1306 → T), rs243865 and TIMP-2 (G303 → A), rs9900972 in the study groups

<table>
<thead>
<tr>
<th>The studied gene</th>
<th>Control group (n=80, %)</th>
<th>Experimental group (with enterocutaneous fistulas) n = 19, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (C-1306 → T)</td>
<td>CC: 38 (47.5%)</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td></td>
<td>CT: 34 (42.5%)</td>
<td>7 (36.8%)</td>
</tr>
<tr>
<td></td>
<td>TT: 8 (10%)</td>
<td>2 (10.5%)</td>
</tr>
<tr>
<td>Hardy-Weinberg test ($\chi^2$, p)</td>
<td>$\chi^2 = 0.01, p &gt; 0.05$</td>
<td>$\chi^2 = 0.21, p &gt; 0.05$</td>
</tr>
<tr>
<td>TIMP-2 (G303 → A)</td>
<td>GG: 50 (50%)</td>
<td>15 (78.9%)</td>
</tr>
<tr>
<td></td>
<td>GA: 32 (40%)</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td></td>
<td>AA: 8 (10%)</td>
<td>0 (%)</td>
</tr>
<tr>
<td>Hardy-Weinberg test ($\chi^2$, p)</td>
<td>$\chi^2 = 0.18, p &gt; 0.05$</td>
<td>$\chi^2 = 0.26, p &gt; 0.05$</td>
</tr>
<tr>
<td>T-test $\chi^2$, ($\chi^2$, p)</td>
<td>-</td>
<td>$\chi^2 = 0.206, p &gt; 0.05$</td>
</tr>
</tbody>
</table>

Thus, the dominant homozygous GG variant was 1.58 times higher than the control values (p = 0.057). Heterozygous GA genotype in the experimental group was twice less common than in the control (21.1% vs. 40%, p = 0.057). Carriers of homozygous AA genotype in the group with enterocutaneous fistulas were not detected, while a similar variant in the control was found in 10% of cases [7].

Thus, as a result of genetic and statistical analysis of gene polymorphism MMP-2 (C-1306 → T) and TIMP-2 (G303 → A) identified genotype variants that are associated with a risk of developing enterocutaneous fistulas.

The differences in allelic variants of the TIMP-2 gene (G303 → A) in groups with enterocutaneous fistulas of anastomoses became the basis for the development of a method for predicting the development of enterocutaneous fistulas, which differs in that detection of GA- and AA-variants, the development of enterocutaneous fistulas is unlikely.

Our proposed method, which includes the genetic study of TIMP-2 gene polymorphism (G303 → A) allows us to predict the likelihood of enterocutaneous fistula, which further requires changes in treatment tactics, improvement of stages and methods of surgery, development of new surgical treatments.
Conclusions

1. The proposed method, which includes genetic analysis of TIMP-2 gene polymorphism (G303 → A), allows predicting the probability of enterocutaneous fistula, which, in turn, will improve the choice of treatment tactics in such patients.

2. 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.

References: