Clinical and molecular genetic parallels of steroid resistance of acantholytic pemphigus

Kutasevych Yanina F.1, Oliinyk Iryna O.2, Abdalla Aliya E-S.E-S.3

1 Doctor of medical sciences, professor, Director SE Institute of Dermatology and Venereology, Chief of Department of dermatology, infectious and parasitic skin diseases; National Academy of Medical Sciences of Ukraine; Ukraine
2 Doctor of medical sciences, Senior Researcher SE Institute of Dermatology and Venereology, Chief researcher of Department of dermatology, infectious and parasitic skin diseases; National Academy of Medical Sciences of Ukraine; Ukraine
3 Phd student SE Institute of Dermatology and Venereology; National Academy of Medical Sciences of Ukraine; Ukraine

Abstract.
The problem of pharmacological resistance is becoming a challenge for doctors of all specialties, especially for people suffering from severe chronic diseases, which lead to disability and death consequences, which are patients on acantholytic pemphigus (AP), who have to take system glucocorticosteroids (SGCS) for a long term. Among the existing hypotheses of formation of steroid resistance (SR) is the perspective study of genetic factors, which are one of the main mechanisms of occurrence of resistance and can be connected with the alleles polymorphism of gene of multidrug resistance (MDR1). The increased expression of this gene leads to the acceleration of the elimination of drugs from cells. The study of molecular genetic peculiarities of SR in patients with AP has not been carried out until now. Thus, the study of polymorphism of MDR1 gene in patients with AP is relevant. The aim. Study the frequency of polymorphism of marker C3435T in gene MDR1 in the relationship with clinical and anamnestic peculiarities of the AP course. Materials and methods. Under the supervision were 33 patients on the AP aged from 29 to 73 years, treated in the dermatological department of the SE "IDV NAMS of Ukraine". The buccal epithelium obtained by scraping mucosa of the oral cavity was examined. The polymorphism of the C3435T marker in the MDR1 gene was determined by the PCR method. The clinical and anamnestic features of patients on AP were evaluated on a specially developed scale. Discussion. The results of the study showed clinical-anamnestic signs of lack of sensitivity to SGCS, which correlated with the results of MDR1 gene expression. The presence in patients with AP in the CT haplotype of the T allele gives reason to classify the group of patients with AP with an index of resistance (IR) as SGCS 7.3 ± 0.6 as a risk group for the development of SR. Conclusion. According to the results of the study in patients with AP, the correlation of clinical-anamnestic signs of SR with the results of gene expression was established MDR1. Defined the risk group for the development of SR by the polymorphism of the MDR1 gene.

Keywords:
acantholytic pemphigus resistance system glucocorticosteroids MDR1 gene polymorphism clinical-anamnestic features of steroid resistance
**Introduction**

One of the most severe dermatological nosologies of autoimmune genesis is AP. Pathogenesis is characterized by the synthesis of autoantibodies to the desmosome of epidermal cells and, as a result, the loss of connections with each other. Clinically, this is manifested by the formation of bullous elements on the skin and mucous membranes [1]. Although the incidence of AP is up to 1% of all dermatological nosologies, the severity of the disease is due to rapid development, general impairment, the development of early and distant complications, a high percentage of disability and deaths [2]. Diagnosis aimed at identifying specific agents and cells helps in establishing a diagnosis, although it does not have 100% sensitivity [3]. Therapeutic tactics are aimed at suppressing the immune response with the help of SGKS and cytostatics, are not perfect due to complications and unpredictable exacerbations [4]. According to the observations of domestic and foreign researchers in recent years, most patients often have recurrent conditions that are poorly exposed to SGCS - SR condition [5].

According to the scientific literature, there are several hypotheses of SR, precisely: a decrease in the level of expression of glucocorticoid (GC) receptors on target cells [6], the formation of defective GC receptor molecules [7], mutations in the gene leading to a violation of the interconnection of GC with the cell nucleus and the transportation of hormone receptor complexes to the target cell nucleus [8].

The main gene that determines the intracellular concentration of substances is the ABCB1 gene (MDR1 - multidrug resistance gene 1). Exactly, the polymorphism of the C3435T marker locus (rs1045642) in the MDR1 gene confirms the hypothesis of a violation of "transport" due to the expression of the protein of the P-glycoprotein group encoded by, and is one of the promising theories for the development of pharmacological resistance [9]. The main function performed by P-glycoprotein is associated with binding to the drug substance entering the cell and removing it to the intercellular space. The gene has a number of allelic forms, but are clinically significant, mainly nucleotide residues in the 26th exon (3435C > T), in the 12th (1236 C > T) and 21st
(2672G > T). The presence of the T allele in the genotype at position 3435 leads to increased expression of P-glycoprotein and withdrawal of drugs from the cell, thereby reducing the therapeutic effect. While the presence of the C allele at position 3435 correlates with the population "baseline" level of gene and protein excretion MDR1 [10].

The scientific literature provides data on the determination of polymorphism of the marker of the C3435T gene of the MDR1 in patients taking anticonvulsive [11], anti-tumor [12], antibacterial drugs [13], as well as in patients with autoimmune diseases of joints and connective tissue receiving immunosuppressive therapy [14]. But these data are contradictory and require further research. The presence of polymorphism in the MDR1 gene in patients with AP receiving SGCS has not been studied until this time.

Therefore, it is relevant to study the polymorphism of C3435T in the gene MDR1 patients with AP to establish the molecular genetic characteristics of SR in this category of patients.

The aim

Study the frequency of polymorphism of marker C3435T in gene MDR1 in the relationship with clinical and anamnestic peculiarities of the AP course.

Materials and methods

Under supervision were 33 patients with AP aged 29 to 73 years, treated in the dermatology department of the SE "IDV NAMS of Ukraine." Diagnosis was made on the basis of clinical signs, cytological examination of the impression smear from the bottom of erosion and/or the bullous element. Assessment of sensitivity to SGCS of patients with AP was determined on a specially developed 12-point scale of IR to SGCS. Signs were determined: lack of therapeutic effect in the first 2 weeks of treatment with high-dose SGCS - 3 points; duration of dermatoses up to a year - 1 point; 2-5 years - 2 points; more than 5 years - 3 points; recurrence rate throughout the year: no relapse - 0 points; 1 relapse - 1 point; up to 2 relapses per year - 2 points; more than 2 relapses - 3 points; prevalence of the process in case of relapse: mucous membranes - 1 point; skin - 1 point; mucous membranes and skin - 3 points. Patients who had 0-4 points were classified as milder course; having 5-8 points - to the group with a medium
current; that had 9-12 points – to the group with a severe course.

The buccal epithelium obtained by scraping mucosa of the oral cavity was examined. The polymorphism of the C3435T marker in the MDR1 gene was determined by the PCR method. Genomic DNA was isolated from the material by a method based on the feedback of nucleic acids to the surface of an inorganic sorbent in the presence of a chaotropic agent (guanidine thiocyanate). Restriction amplification of DNA fragments for C3435T was carried out by adding 5 μl of restriction mixture to each amplification product tube at 37 °C for one hour on GeneAmp PCR System 9700 (AppliedBioSystems). The next step was to distribute restriction products by horizontal gel electrophoresis in a 2% agarose gel which included ethidium bromides. SSCP electrophoresis was carried out for one hour at a voltage of 120 V. According to the results of the separation of restriction products of the MDR1 gene fragment with the existing mutation, fragments were C3435T formed, which made it possible to clearly differentiate allelic variations in CC, CT, TT. Confirmation of the results of SSCP electrophoresis was carried out using two-way sequencing by ABI Prism 3130x1, AppliedBioSystems [15].

The data were statistically processed using Statistica 12.6. To compare frequencies, the Pearson χ² test was used, in the case of a small sample (no more than 10 cases), the Yates's correction test was used, significant differences were considered at p < 0.05. Using the non-parametric Mann-Whitney test, significant differences in scores between groups were found. Differences were considered significant at p < 0.05 [16].

**Results and discussion**

Under supervision in the dermatology department of the SE "IDV NAMS of Ukraine" there were 33 patients with AP. The age of patients ranged from 29 to 73 years. Women made up 27 people (81.8%), men – 6 people (18.2%). The control group included 20 practically healthy persons representative by age and sex.

According to the clinical history of the disease, patients were divided into two groups: steroid sensitive (SS) -10 people (30.3%) and SR - 23 people (69.7%). At the same time,
the assessment was carried out on a specially developed IR to SGCS point scale. Signs were determined: lack of therapeutic effect in the first 2 weeks of treatment with high-dose SGCS - 3 points; duration of dermatoses up to a year - 1 point; 2-5 years - 2 points; more than 5 years - 3 points; recurrence rate throughout the year: no relapse - 0 points; 1 relapse - 1 point; up to 2 relapses per year - 2 points; more than 2 relapses - 3 points; prevalence of the process in case of relapse: mucous membranes - 1 point; skin - 1 point; mucous membranes and skin - 3 points. Patients who had 0-4 points were classified as milder course; having 5-8 points - to the group with a medium current; that had 9-12 points - to the group with a severe course. When evaluating the data obtained, it was found that the IR to SGCS in patients with group I ranged from 3.6 ± 0.3 to 6.3 ± 2.2 points and averaged 4.4 ± 0.7 points. In patients with group II - from 7.5 ± 0.6 points to 9.2 ± 0.4 points, on average equal to 8.2 ± 0.4, and significantly different from group I persons (Tab. 1).

Table 1

<table>
<thead>
<tr>
<th>GENE</th>
<th>ALLELE VARIATION</th>
<th>I GROUP (SS)</th>
<th>II GROUP (SR)</th>
<th>( \chi^2; p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>IR TO SGCS (POINTS)</td>
<td>N (%)</td>
<td>IR TO SGCS (POINTS)</td>
</tr>
<tr>
<td>TT</td>
<td>0 (0%)</td>
<td>-</td>
<td>9 (27,3%)</td>
<td>9,2±0,4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>3 (9,1%)</td>
<td>6,3±2,2</td>
<td>14 (42,4%)</td>
<td>7,5±0,6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>7 (21,2%)</td>
<td>3,6±0,3</td>
<td>0 (0%)</td>
<td>-</td>
</tr>
<tr>
<td>ALL GROUPS</td>
<td>10 (30,3%)</td>
<td>4,4±0,7</td>
<td>23 (69,7%)</td>
<td>8,2±0,4</td>
</tr>
</tbody>
</table>

\( \chi^2; p - to compare case rates. \)
Mann-Whitney U test (U; p) to compare points

According to the indicators of IR to SGCS, patients of group I were classified as SS, patients of group II - as SR.

The view on that the main gene that determines the intracellular concentration of substances is the MDR1 gene, which is responsible for binding to the drug that entered the cell and removing it to the intercellular space. The presence of the T allele in the genotype at position 3435 leads to increased excretion of drugs from the cell, which reduces the therapeutic effect. While the presence of the C allele at the 3435 position correlates with the population "baseline" level of gene and protein excretion MDR1 [10]. In view of the above mentioned, we conducted a study on genetic belonging of patients to a certain genetic type, with their further comparison with clinical-anamnestic conditions.

To determine the incidence of allele variation among patients and relatively healthy individuals, the variation of polymorphism alleles in the MDR1 gene in patients with AP and controls was verified. According to comparative analysis, the allele variation was as follows: homozygous polymorphism according to the SS haplotype in 21.2% (7 people), according to the TT haplotype in 27.3% (9 people) and according to the heterozygous variation of alleles according to the ST haplotype - in 51.5% (17 people) in patients with AP. Allele variation in the CC control group is 20% (4 persons), TT is 35% (7 persons), CT is 45% (9 persons) (Fig.1).

<table>
<thead>
<tr>
<th>PATIENTS WITH AP</th>
<th>CONTROL GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT; 27,3%</td>
<td>CC; 21,2%</td>
</tr>
<tr>
<td>CT; 51,5%</td>
<td></td>
</tr>
<tr>
<td>n=33 (CC-7; CT-17; TT-9)</td>
<td>n=20 (CC-4; CT-9; TT-7)</td>
</tr>
</tbody>
</table>

Figure 1
Association of haplotypes of C3435T marker polymorphism (rs1045642) of the MDR1 gene in patients with AP and control group.
Therefore, these frequencies of occurrence of allelic variation in patients with AP and control groups did not have a significant difference and on average coincided with the distribution of allelic variation frequencies among the population of Ukraine (according to Levkovich N.M.): SS – 22.37%, ST – 50.88%, TT – 26.75%, which is indicated in scientific literary publications [17].

Statistical parametric analysis of Pearson's $\chi^2$ coefficient was used to determine the accuracy of polymorphism frequencies in the MDR1 gene in patients with AP and in a group of healthy individuals (Tab. 2).

Table 2

<table>
<thead>
<tr>
<th>ALLELE VARIATION</th>
<th>PATIENTS WITH AP</th>
<th>CONTROL GROUP</th>
<th>$\chi^2$; P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>TT</td>
<td>9</td>
<td>27.3%</td>
<td>7</td>
</tr>
<tr>
<td>CT</td>
<td>17</td>
<td>51.5%</td>
<td>9</td>
</tr>
<tr>
<td>CC</td>
<td>7</td>
<td>21.2%</td>
<td>4</td>
</tr>
</tbody>
</table>

It was found that the incidence of polymorphism in the MDR1 gene in patients with AP and a group of healthy individuals did not have a reliably significant difference (TT – $\chi^2 = 0.4$, p = 0.6; CT – $\chi^2 = 0.2$, p = 0.6; CC – $\chi^2 = 0.01$, p = 0.9).

Also, the frequency of occurrence of the polymorphism of the C3435T marker in the MDR1 gene in patients with AP, depending on the belonging to the group, was determined. Calculations were carried out using the Pearson $\chi^2$ Yates correction test (Table 1).

The frequency of occurrence of the polymorphism of the MDR1 gene with a variation of TT and CT alleles (groups I and II), in the ratio of 0:9 to 3:14, did not have a reliably significant difference $\chi^2_{TT-CT} = 1.8$; p = 0.18. The ratio of detection frequencies of allelic variations of TT and SS (in both groups) in the ratio of 0:9 to 7:0 with a high probability had reliably significant differences – $\chi^2_{TT-CC} = 12.2$;
p = 0.0005. In terms of the frequency of occurrence of allelic variations in ST and CC, both groups, in the ratio of 3:14 to 7:0, reliably significant differences were observed, having a value of $\chi^2_{\text{ST-CC}} = 10.65; p = 0.001$.

Given the data of the scientific literature and the results of the study, the main gene that determines drug resistance is the genotype T. It was found that the TT haplotype was detected in 9 people (27.3%) and the T allele of the ST genotype locus in 17 people (51.5%) of the gene were MDR1 dominant in the group of examined patients and indicated resistance to SGCS.

In patients with IR to SGCS 9.2 ± 0.4 points, having a homozygous TT allele and a severe degree of AP, the condition of patients was assessed as SR. In patients with index 3.6 ± 0.3 with homozygous allele according to the CC haplotype, the condition was considered mild as SS. The parameters of IR to SGCS in the patients of the studied groups were significantly different from each other, which was proved by mathematical statements (Tab. 1).

In 17 people with AP, the average IR to SGCS value was 7.3 ± 0.6 points, heterozygous alleles were found according to the CT haplotype: in 3 people (9.1%) from the SS group, whose IR to SGCS was 6.3 ± 2.2 points; in 14 people (47.4%) - from the SR group, whose IR to SGCS was equal to 7.5 ± 0.6 points. The course of the disease in these patients was characterized as moderate. No statistical difference in IR to SGCS ($U_{\text{ST-CC}} = 6.5; p = 0.36$).

IR to SGCS, taking into account clinical and anamnestic signs 7.3 ± 0.6 points, the presence of the T allele in the haplotype of CT, which leads to accelerated withdrawal of drugs from cells and reduces the therapeutic effect, gives reason to classify patients with the above characteristics as at risk for the development of resistance to SGCS.

Conclusion

In determining the clinical-anamnestic features of the course of AP with the help of a specially developed scale, patients I of the groups with average IR scores in the SGCS 4.4 ± 0.7 were classified as SS; in the second group of patients, the average value of IR to SGCS in which was almost twice as large and equal to 9.2 ± 0.4 was classified as SR.
According to the distribution of frequencies of occurrence, the variation of alleles of the C3435T marker in the MDR1 gene among patients with AP and the control group of persons did not have a reliable difference and on average coincided with the data of population indicators among the population of Ukraine.

Based on the analysis of the frequencies of occurrence of allelic variations of the C3435T marker polymorphism in the MDR1 gene, in patients with AP, with IR to SGCS of 9.2 ± 0.4 points, a severe degree of disease course was observed, and the TT allele was homozygous in 27.3% (9 people) of cases. Patients with a mild degree in 21.2% (7 people) had IP to SGCS 3.6 ± 0.3 points and allelic variation of CC. With an average IR to SGCS value of 7.3 ± 0.6 points in 51.5% (17 people), which included 9.1% (3 people) from the SS group and 42.4% (14 people) from the SR groups, the general condition of which was considered as moderate, the presence of a heterozygous ST allele gave reason to classify patients as at risk group.

Thus, the presence of the T allele in patients with AP in the CT haplotype gives reason to classify the group of patients with AP with IR as SGCS 7.3 ± 0.6 as a risk group for the development of resistance to SGCS, which will make it possible to adjust therapy for each patient individually in the early period and prevent the development of severe complications.

References: