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The Effectiveness Of Pharmacotherapy For Dmards With Ra Depending On The C3435t Polymorphism Of The Mdr1 Gene

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Annotation: ***The article discusses data on the effectiveness of pharmacotherapy for DMARDs in RA depending on the C3435T polymorphism of the MDR1 gene. It is known that genetic features arise due to nucleotide substitutions in the DNA molecule, which are involved in different ways in the pharmacodynamics or pharmacokinetics of drugs. The identification of such substitutions allows predicting the pharmacological response and, therefore, a personalized approach to the choice of the drug and its dose. The data obtained indicate that the heterozygous carriage of MDR1 was associated with a large decrease in the CDAI index by 1.29 times compared with the carriage of the homozygous allelic variant. In heterozygous carriers of alleles, the frequency of a decrease in the overall assessment of disease activity in patients according to the VAS was significantly higher by 1.05 times compared with carriers of normal allelic variants.***

Key words: pharmacogenetics, methotrexate, glycoprotein, MDR1, allele, polymorphism

1. INTRODUCTION

Precently, pharmacogenetic testing is the most promising for clinical practice and one of the applied tools of personalized medicine. Pharmacogenetic testing permits to determine the effectiveness and safety of therapy, to appoint the most effective drug as the primary therapeutic agent, which leads to a decrease in the number of drugs required for adequate treatment.

Pharmacogenetic testing analyzes changes in the pharmacological response associated with the genetic characteristics of patients. Genetic features arise from nucleotide substitutions in the DNA molecule, which are involved in different ways in the pharmacodynamics or pharmacokinetics of drugs. The identification of such substitutions allows predicting the pharmacological response and, therefore, a personalized approach to the choice of the drug and its dose. It is considered expedient to use pharmacogenetic testing when the frequency of occurrence of polymorphisms of the studied gene is more than 20% of cases [2, 5].

It is now becoming clear that the pharmacological response in patients with the same disease will not be the same, but will depend on the genotype of each individual. Currently, the concept of personalization of those drugs that were previously considered universal is gaining momentum. Taking into account the complex approach to the treatment of patients with RA and the ongoing individualization of therapy, the study of pharmacogenetic aspects is necessary to increase the effectiveness of treatment and increase its safety [15].

Enzymes that provide the pharmacokinetic functions of absorption, distribution and excretion of drugs from the body are called "drug transporters". These include glycoprotein P,

transporters of organic anions and cations, etc. Of greatest interest from a pharmacogenetic point of view is the MDR1 gene polymorphism encoding glycoprotein P (locus 7q21.1). This enzyme controls the release of various xenobiotics from the cell, prevents the absorption of drugs from the intestine. Specific glycoprotein-P (Pgp) plays the role of a pump that ejects many foreign substances from the cell, including drugs the important role of Pgp in the development of drug resistance is well known in oncology [3]. In rheumatology, this concept has not yet received application, despite the widespread use of anticancer drugs as basic therapy. The substrates of glycoprotein P are cardiac glycosides, slow calcium channel blockers, statins, macrolides, cytostatics, antiviral drugs [4]. One of the most common drugs used to treat this condition is methotrexate because it is less expensive, effective, and safe to use. This drug can be taken with many other disease-modifying antirheumatic drugs, but it has been observed that approximately 30% of patients with rheumatoid arthritis did not respond to methotrexate [12]. Since methotrexate is involved in many drug pathways, even though it is a small drug molecule, its metabolism is difficult. In a patient with rheumatoid arthritis, the mechanism of action of methotrexate depends on the endogenous release of adenosine. Metabolism of methotrexate is associated with gene polymorphism at different stages, for example, in the adenosine pathways and during the movement of cellular enzymes in folic acid.

Thus, the study of the mechanism of pharmacogenetic characteristics of basic anti-inflammatory drugs requires further more detailed study, which will contribute to the effectiveness of therapy and reduce the risk of developing adverse drug reactions in patients suffering from immuno-inflammatory diseases.

The aim of the study was to study the efficacy of basic therapy for RA with methotrexate in patients with various allelic variants of the MDR1 gene.

2. MATERIAL AND RESEARCH METHODS.

The present research is based on the results of observation of patients with rheumatoid arthritis (RA) hospitalized from 2019-2021 in the departments of rheumatology of the clinic No. 1 of SamSMI. There were 117 patients with the diagnosis of RA established on the basis of anamnesis, physical and clinical laboratory studies according to the criteria of the American Rheumatological Association (ARA) and the working classification proposed by the Russian Association of Rheumatologists.

The age of study population ranged from 30 to 75 years (mean age 56.14 ± 11.69 years), with a disease duration from 3 months up to 30 years (average duration 9.85 ± 3.44 years). Rheumatoid factor (RF) was detected in 35 patients, and the remaining 20 patients were seronegative. 18 patients had RA with systemic manifestations, and 37 had an articular form. 32 patients had a slowly progressive course of the disease, and the remaining 23 had a rapidly progressive course.

Obtaining biosamples for molecular genetic research methods

Blood was taken from the cubital vein in a horizontal position, in the morning, on an empty stomach (at least 9 hours). To obtain plasma, blood (3 ml) was collected in a disposable tube (BD Vacutainer, Plymouth, UK) containing an anticoagulant agent (trisodium citrate).

Immediately after receiving the sample was centrifuged for 15 minutes (3000-4000 rpm, 4 ° C), plasma was obtained, aliquoted into labeled secondary plastic Eppendorf tubes and placed in a -20 ° C freezer.

Determination of polymorphism of the studied genes
The material for DNA isolation was venous blood from the cubital vein with a volume of 3-5 ml (Beckton-Dickinson vacutainers were used for blood sampling) with an anticoagulant / preservative 15% tripotassium EDTA (Ethilendianin-tetraaceticacid). Blood for further processing could be stored for up to 24 hours at a temperature not exceeding + 4 ° C.

Polymerase chain reaction (PCR) was performed on a Rotor-Gene-2000 thermal cycler (CorbettResearch) using appropriate primers and 10 μl PCR mixture (NPO Litekh) containing 2 mM MgCl2, Taq DNA polymerase and Cresol red dye. The results were visualized by electrophoresis in a 2% agarose gel with ethidium bromide at 150 V and 290 mA. The size of the fragments was determined using the standard for the size of the lengths of DNA fragments from Life Technologies.

Polymerase chain reaction (PCR)
To detect the studied polymorphisms, amplification of certain regions of the corresponding genes was carried out. To determine the polymorphic alleles of genes, the restriction fragment length polymorphism (RFLP) method was used.

Determination of polymorphism (C3435T) of the MDR1 gene was performed by PCR-RFLP. After a hot start and the first denaturation (94 ° C, 2 min), amplification was carried out in 32 cycles, each of which included: denaturation (94 ° C, 30 sec), primer annealing (55 ° C, 1 min), elongation (74 ° C, 1 min). PCR was carried out in a mixture with a volume of 25 μL, which included 10 pmol of each primer, 200 μM of each dNTP, 1 μL (about 50 ng) of genomic DNA, and a PCR mixture from Interlabservice. 8.5 μl of a 306 bp PCR product incubated at 37 ° C with 0.5 μl (3 U) of restriction enzyme Ava-I and 1 μl of 10-fold buffer for restriction. Restriction products were visualized by electrophoresis in a 2% agarose gel containing 1 μg / ml ethidium bromide. At position C3435T of the MDR1 gene, the C-allele contains a restriction site and is detected by the presence of two fragments 190 and 116 bp, the T-allele does not contain a restriction site, the fragment is 306 bp.
Currently, in routine practice and in clinical trials, three total indices of inflammatory activity are widely used - DAS28, SDAI, and CDAI [6].They all have their merits and demerits, and none of them are considered the gold standard. The CDAI index is calculated according to 4 clinical parameters: CDAI = DAD + DAP + NSJ + NPJ (where DAD is the general assessment of the disease activity by the doctor according to VAS (cm), DAP is the overall assessment of the disease activity in patients according to VAS (cm), NSJ is the number swollen joints out of 28, NPJ - the number of painful joints out of 28). CDAI> 22 was suggested to be considered a sign of high, 22≤CDAI <10 - moderate, 10≤CDAI <2.8 - low RA activity. Remission corresponds to CDAI≤2.8 [7]. At the same time, the authors consider CRR to be a significant indicator and propose to take into account its value in addition to the index. According to current clinical guidelines, it seems appropriate to conduct laboratory examination of acute phase parameters - ESR and CRP in all patients with RA in order to initiate therapy and identify potential risk factors for ADR before prescribing antirheumatic therapy (all groups of drugs), as well as monitoring the efficacy and safety of drug therapy [13].

An assessment was made of the difference between the CDAI index, which is calculated according to 4 clinical parameters (CDAI = DAD + DAP + NSJ + NPJ), indicators of acute phase parameters - ESR and CRP, before taking DMARDs with such symptoms in patients after 3 months of treatment.

3. RESEARCH RESULTS

To assess the effect on the success of therapy in patients with RA, we evaluated the difference between the CDAI index before taking methotrexate in the hospital with the patient's CDAI index after 3 months of treatment. Patients receiving methotrexate were divided into 2 subgroups depending on the studied polymorphic allelic variant of the MDR1 gene.
The results of the difference in CDAI indices in patients with different allelic variants of the MDR1 gene are presented in Table 1.

Table 1
Difference in CDAI indices before methotrexate administration and after 3 months of treatment, depending on the allelic variant of the MDR1

Gene	Alleles	Index before methotrexate administration CDAI	Index difference
MDRI C3435T (rs 1045642)	C\C	17,41±4,11	5,58±0,08
	C\T	18,55±3,81	4,31±0,03*

From the results obtained, it can be seen that the heterozygous carriage of MDR1 is associated with a large decrease in the CDAI index by 1.29 times compared with the carriage of the homozygous allelic variant. Moreover, a greater decrease in the CDAI index in heterozygous carriage is statistically significantly different from the homozygous allelic variant.
Further, we also calculated the difference between each clinical parameter of the CDAI index before taking methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene.
We calculated the difference between the general assessment of the disease activity by the doctor (DAD) according to the VAS before taking methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene.

Table 2
The difference in DAD before the appointment of methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene.

Gene	Alleles	DAD before prescribing methotrexate	DAD difference
MDRI C3435T (rs 1045642)	C\C	6,54±1,02	2,42±0,03
	C\T	6,61±0,92	2,21±0,08*

From the results obtained, it can be found that heterozygous carriage is associated with a large decrease in the general assessment of the disease activity by the doctor according to the VAS by 1.09 times compared with the carriage of the homozygous allelic variant.

Table 3
The difference in DAP before the appointment of methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene.

Gene	Alleles	DAP before prescribing methotrexate	DAP Difference
MDRI C3435T (rs 1045642)	C\C	7,91±1,14	3,73±0,04
	C\T	7,88±1,31	3,54±0,06*

The results of the difference in the overall assessment of disease activity in patients according to the VAS in patients with different allelic variants of the MDR1 gene are presented in Table 3. The presented results demonstrate that in heterozygous carriers of alleles, the frequency of a decrease in the overall assessment of the activity of the disease in patients according to the VAS is significantly higher by 1.05 times compared with carriers of normal allelic variants.

We also studied the effectiveness of basic therapy for the number of swollen joints out of 28 fixed doses of methotrexate in patients with different allelic variants of the MDR1 gene. So, we calculated the difference between the number of swollen joints from 28 before taking methotrexate and after 3 months of treatment of the disease.

Table 4
The difference in NSJ before the appointment of methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene.

Gene	Alleles	NSJ before prescribing methotrexate	NSJ Difference
MDRI C3435T (rs 1045642)	C\C	14,45±1,21	6,57±0,07
	C\T	15,21±0,98	6,45±0,04*

After a course of treatment with methotrexate, a significant decrease in the number of swollen joints out of 28 from 15.21 to 6.45 was recorded in the group of patients with heterozygous carriage of alleles.
The results of the difference in the number of painful joints out of 28 in patients with different allelic variants of the MDR1 gene are presented in Table 5. The presented results demonstrate that in heterozygous carriers of alleles, the frequency of a decrease in the number of painful joints out of 28 is significantly higher by 1.15 times compared with carriers of normal allelic variants.

Table 5
Difference in NPJ before the appointment of methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene

Gene	Alleles	NPJ before prescribing methotrexate	NPJ Difference
MDRI C3435T (rs 1045642)	C\C	14,84±2,24	8,55±0,77
	C\T	14,11±1,46	7,4±0,63

Thus, the analyzed clinical parameters of the CDAI index by the end of 3 months of treatment with methotrexate more regressed in patients with heterozygous carriage of alleles: DAD - from 6.61 to 2.21 points; DAP - from 7.88 to 3.54 points; NPV - from 15.21 to 6.45 points and NPV - from 14.11 to 7.4 points.

ESR is a highly sensitive, but non-specific and unstable marker of systemic inflammation. The results of determining ESR are influenced by age, gender, fibrinogen level, RF, hypergammaglobulinemia, anemia, and other factors. In early RA, increased ESR correlates with disease activity and the risk of progression of joint destruction [13].
To identify the effect of basic pharmacotherapy with methotrexate on the level of acute phase parameters, we monitored changes in the concentration of ESR and CRP in patients with homozygous and heterozygous allelic variants. The results are shown in Tables 6-7.

Table 6
The difference in ESR before the appointment of methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene

Gene	Alleles	ESR before prescribing methotrexate	ESR Difference
MDRI C3435T (rs 1045642)	C\C	46,11±0,78	32,61±1,41
	C\T	47,51±1,04	30,9±1,27

In individuals with different allelic variants of the MDR1 gene, significant changes in ESR were found during the treatment period with the inclusion of methotrexate in the basic pharmacotherapy. A decrease in concentration was recorded in cases with the carriage of the allelic CC variant, and a more pronounced decrease was associated in cases with the carriage of the allelic CT variant of the MDR1 gene.

CRP is a classic serum acute phase protein considered to be the most sensitive laboratory biomarker of inflammation, infection, and tissue damage. Determination of CRP is used to predict the rate of joint destruction and differential diagnosis of RA. CRP is a more stable and reproducible biomarker of inflammation than ESR. ESR and CRP are among the RA classification criteria (ACR / EULAR, 2010), and are components of the RA activity indices.

Table 7
The difference in CRP before the appointment of methotrexate and after 3 months of treatment, depending on the allelic variant of the MDR1 gene

Gene	Alleles	CRP before prescribing methotrexate	CRP Difference
MDRI C3435T (rs 1045642)	C\C	57,14±1,25	37,84±3,21
	C\T	57,81±1,73	32,31±2,42

When studying CRP during treatment with methotrexate in patients, depending on the allelic variant of the MDR1 gene, it was found that a significant decrease in the level of the studied cytokines is observed in patients with the CT allele, and in the presence of an allelic variant

of the MDR1 gene in the CC genotype, changes in the indicator of this acute phase protein are insignificant.

Therefore, it can be assumed that the C / T allele in the genotype of RA patients is accompanied by a more pronounced pharmacodynamic effect of MT on the synthesis of acute phase parameters - ESR and CRP, which, in turn, determine the development of the systemic immune-inflammatory process and, in particular, the pathological process of the joints.

Thus, MT makes it possible to effectively control clinical symptoms (DAD, DAP, NPV, ChBS), to improve the acute phase parameters of inflammation, due to its mechanism of action. MT may be the first choice among DMARDs for the treatment of RA in the presence of the C / T allele of the MDR1 gene in the genotype of patients.

The present work has provided evidence for the principle that biomarkers can be identified to predict response to basic pharmacotherapy. This biomarker allows for a better understanding of the mechanism that contributes to the efficacy and safety of methotrexate, as well as the mechanism of action of treatment.

MDR1 is localized to the apical membrane of many epithelial, endothelial cells, and lymphocytes. Plays a key role in the absorption and distribution of methotrexate in the body [16]. One of the most significant single nucleotide polymorphisms of the ABCB1 gene is the substitution of cytosine thymine at the 3435 position (rs1045642 / C3435T) [8]. It is believed that it may be associated with both the severity of toxicity and the effectiveness of methotrexate in the treatment of rheumatoid arthritis. M.C. deRotte et al. noted an increase in the effectiveness of methotrexate in carriers of this mutation. According to scientists, this is due to a violation of the excretion of methotrexate [10]. At the same time, it is known that MDR1, localized on the cell membrane of CD4 and CD8 lymphocytes, NK cells and their precursors, regulates the transport of a number of inflammatory mediators, in particular, bioactive lipids. A decrease in MDR1 activity associated with the C345T mutation can reduce the transport of inflammatory mediators and, as a consequence, contribute to the development of a good therapeutic effect of methotrexate [11]. A number of pharmacogenetic studies have demonstrated an increase in toxicity in T-homozygous patients [9]. J.C. Plaza-Plaza et al. Noted a trend towards the development of undesirable reactions to carriers of allele C [14].

Currently, according to the recommendations of the European League Against Rheumatism (EULAR) [1], methotrexate is considered the "gold standard" for the treatment of rheumatoid and psoriatic arthritis, due to its high efficacy, good tolerability and ease of administration. For the basic treatment of rheumatoid arthritis, methotrexate is taken only once a week. For the first time, a specific day of the week is chosen, and from then on, throughout the course of treatment, methotrexate is taken only on that day.

The decision can be influenced by patient-specific characteristics, such as advanced age and the presence of impaired renal, hepatic, hematological disorders. The MT appointment should be individualized. The drug of first choice among DMARDs for the treatment of RA in the presence of the C / T and T / T alleles of the MDR1 gene in the genotype of patients is MT. MT treatment should be started with a dose of 15 mg / week. On the background of MT treatment, it is recommended to take folic acid, which should be prescribed no earlier than 24 hours after MT intake. For patients with active RA in the presence of the C / C allele of the MDR1 gene in the genotype, it is advisable to prescribe a combination therapy with MT and other standard therapy in combination with HA or without HA. Patients with "active" RA (22≤CDAI <10) Genotyping MDR1 C3435T (rs1045642) Carriage of C / T and T / T genotypes. Carriage of C / C genotype

If the patient is a carrier of the C / T and T / T genotypes of the MDR1 gene, treatment should be started with MT with a dose of 15 mg / week, if the patient is a carrier of the C / C

genotypes of the MDR1 gene, it is recommended to switch to combination therapy of MT with other standard DMARDs in combination with or without GC.

4. CONCLUSION:

The C3435T (rs1045642) polymorphism in the MDR1 gene is associated with RA. Allele T and a combination of CT + TT genotypes of MDR1 polymorphism (OR = 0.45; 95% CI, 0.25> 0.451> 0.814 and OR = 1.96; 95% CI, 0.994> 1.964> 3.879, respectively) are markers of an increased risk of developing RA. The C677T polymorphism in the MTHFR gene is not associated with RA in a sample of patients from the Uzbek population. An increase in the frequency of MDR1 + 3435TT and a decrease in the frequency of genotypes MDR1 + 3435CC were revealed in patients with seropositive RA as compared with the subgroup of seronegative ones. When comparing groups of patients based on the status of ACCP for the polymorphic marker C677T in the MTHFR gene, no significant differences were found. The presence of C / T alleles in the genotype of patients with rheumatoid arthritis is accompanied by a more pronounced pharmacodynamic effect of MT on the synthesis of acute phase parameters - ESR and CRP, which, in turn , determine the development of the systemic immune-inflammatory process and, in particular, the pathological process of the joints.
The presence of C / T alleles in the genotype of patients with rheumatoid arthritis is accompanied by a more pronounced pharmacodynamic effect of MT on the synthesis of acute phase indicators - ESR and CRP, which, in turn, determine the development of the systemic immune-inflammatory process and, in particular, the pathological process of the joints.

5. REFERENCES:

[1] Beketova, T.V. (2019). Review of the European League Against Rheumatica (EULAR) recommendations reviewed in 2018 Modern Rheumatology, 13 (2)
[2] Kantemirova, B.I. (2012). The state of pharmacogenetic research in the Russian Federation and abroad. Astrakhan Medical Journal, 7 (4),
[3] Kolosova, I.R. (2003). Multidrug resistance factor glycoprotein R in rheumatoid arthritis. Scientific and practical rheumatology, (1)
[4] Kukes, V.G. (2010). Personalized medicine in clinical pharmacology. Biomedicine, (3)
[5] Kukes, V.G., Sychev, D.L., & Ignatiev, I.V. (2006). Clinical Pharmacogenetics and Practical Health Care: Prospects for Integration. Biomedicine, (5).
[6] Sychev, D.A., & Kukes, V.G. (2007). Clinical pharmacogenetics., Kukes, V.G., Sychev, D.A., Ramenskaya, G.V., & Ignatiev, I.V. (2007). Pharmacogenetics of the biotransformation system and drug transporters: from theory to practice
[7] Aletaha D., Smolen J. The Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI): a review of their usefulness and validity in rheumatoid arthritis // ClinExpRheumatol. 2005. Vol. 23 (5 Suppl 39). S. 100-108.
[8] Ameyaw M. M., Regateiro F., Li T. et al. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity // Pharmacogenetics. 2001. Vol. 11.No. 3. P. 217-221.
[9] . Bohanec Grabar P., Logar D., Lestan B. et al. Genetic determinants of methotrexate toxicity in rheumatoid arthritis patients: a study of polymorphisms affecting methotrexate transport and folate metabolism // Eur. J. Clin. Pharmacol. 2008. Vol. 64. No. 11. P. 1057-1068.

[10] De Rotte M.C., Bulatovic M., Heijstek M.W. et al. ABCB1 and ABCC3 gene polymorphisms are associated with first-year response to methotrexate in juvenile idiopathic arthritis // J. Rheumatol. 2012. Vol. 39. No. 10. P. 2032–2040.
[11] Drozdzik M., Rudas T., Pawlik A. et al. The effect of 3435C> T MDR1 gene polymorphism on rheumatoid arthritis treatment with disease-modifying antirheumatic drugs // Eur. J. Clin. Pharmacol. 2006. Vol. 62. No. 11. P. 933-937.
[12] Korman, B.D., Kastner, D.L., Gregersen, P.K. et al. STAT4: Genetics, mechanisms, and implications for autoimmunity. CurrAllergyAsthmaRep 8, 398-403 (2008).).
[13] Nasonov, E. L. (2017). The 2016 EULAR guidelines for the diagnosis and treatment of early arthritis. Rheumatology Science and Practice, 55 (2), 138-150.
[14] Plaza-Plaza J. C., Aguilera M., Canadas-Garre M. et al. Pharmacogenetic polymorphisms contributing to toxicity induced by methotrexate in the southern Spanish population with rheumatoid arthritis // OMICS. 2012. Vol. 16.No. 11. P. 589–595.
[15] Qamar, T., & Mukherjee, S. (2021). Genetic approaches for the diagnosis and treatment of rheumatoid arthritis through personalized medicine. Gene Reports, 23, 101173
[16] Stamp L.K., Chapman P.T., O'Donnell J.L. et al. Polymorphisms within the folate pathway predict folate concentrations but are not associated with disease activity in rheumatoid arthritis patients on methotrexate // Pharmacogenet. Genomics. 2010. Vol. 20. № 6. P. 367–376