Studying the Association of MTRR (rs1801394) Genetic Polymorphism with the risk of Developing Rheumatoid Arthritis in Uzbekistan

Marif Sh. Karimov	Karimov Marif Shakirovich – Doctor of Medical Sciences, Professor, Head of the department of propaedeutics internal diseases, Tashkent medical academy;
Aida A. Eshmurzayeva	Eshmurzayeva Aida Abduganiyevna. - DSc, Associate Professor of the department of propaedeutics internal diseases, Tashkent medical academy
Fazilat N. Shukurova	Shukurova Fazilat Narmamatovna – PhD, associate professor of the department of propaedeutics internal diseases, Tashkent medical academy, Tashkent, Uzbekistan Email: fazilatshukurova@gmail.com +998973330686
Marina V. Sibirkina	Sibirkina Marina Valerovna - PhD, associate professor of the department of propaedeutics internal diseases, Tashkent medical academy;
Asiya R. Gimadutdinova	Gimadutdinova Asiya Rinatovna - assistant of the department of propaedeutics internal diseases, Tashkent medical academy;

ABSTRACT

We have studied the association of MTRR polymorphism (rs1801394) with the risk of developing rheumatoid arthritis among the population of Uzbekistan. The assessment of this genetic polymorphism was carried out by analyzing DNA samples by standard PCR. The results of the study we obtained made it possible to establish the correspondence between the differences between the expected and observed frequencies of the genotype variants of the MTRR (rs1801394) gene polymorphism for RHB ($p>0.05$) both in the group of patients and in the comparison group. The proportion of occurrence of unfavorable allele (G) and genotypes (A/G and G/G) in the general group of patients with RA and among patients with the articular form of the disease does not significantly differ from those in the control ($\chi2<3.84$; $P>0.05$). Only among patients with the articular-visceral form, there is a tendency to increase the occurrence of the A/G genotype by 1.6 times ($\chi2=1.3$; $P=0.262$; OR=1.6; 95% CI: 0.716-3.504). Perhaps, with a larger coverage of the examined RA, a significant relationship with the development of the articular-visceral form of the disease could be established.

Keywords: rheumatoid arthritis, MTRR genetic polymorphism (rs1801394), allele, frequency, genotype, carrier proportion, articular form of rheumatoid arthritis, articular-visceral form of rheumatoid arthritis.

Relevance. Rheumatoid arthritis (RA) is a very complex autoimmune multifactorial disease with a completely unexplored pathogenesis [1,5,6,10]. At the same time, over the past decades, molecular genetic studies of leading specialists in the field of rheumatology have shown that polymorphic variants of folate metabolism genes play a special role in the implementation of the mechanisms of RA formation [3,7,8]. In this regard, polymorphic variants of the MTRR gene located on chromosome 5p15.3 and encoding such a

cytoplasmic enzyme as methionine synthase reductase, converting homocysteine back into methionine, are of particular interest [4]. Changes in the structure of the MTRR gene lead to the formation of its various polymorphic variants, which is accompanied by disturbances in the activity of the gene and the launch of complex pathological processes, which are the basis for the development of RA [9].

There is information in the literature about the degree of involvement of the MTRR gene polymorphism in the mechanisms of RA formation [7,8]. At the same time, if the results of some studies indicate its pathogenetic role in the development of RA [3], others show the absence of a connection between it and the disease [8]. This served as the basis for our research to assess the contribution of the A66G (rs1801394) polymorphism of the MTRR gene to the development of RA in Uzbekistan.

Material and methods. The present study was conducted among 106 RA patients (general group of RA patients), unrelated patients and 109 conditionally healthy individuals without a history of autoimmune diseases living in the territory of the Republic of Uzbekistan. All patients with RA were treated at the 3rd clinic of the Tashkent Medical Academy (2018-2021), where the diagnosis was made based on the ACR/EULAR criteria (2010) [2].

To assess the contribution of the A66G (rs1801394) polymorphism of the MTRR gene to the development of one or another form of RA, patients (n=106), depending on the form of the disease, were divided into two subgroups 1A (n=74, articular form of RA) and 1B (n=32, articular - visceral form of RA).

Molecular genetic studies were carried out in the laboratory of medical genetics on the basis of the Republican Specialized Scientific and Practical Medical Center for Hematology (Republic of Uzbekistan, Tashkent). To do this, DNA was isolated from blood leukocytes, followed by SNP-PCR analysis of the A66G (rs1801394) variant of the MTRR gene using the Applied Biosystems 2720 system (USA) and using test systems from LITEX (Russia). The results were analyzed using the statistical software package "OpenEpi 2009, Version 9.3".

Results and discussion. An analysis of the distribution of the expected and observed frequencies of the genotypes of the A66G (rs1801394) polymorphism of the MTRR gene with an assessment of compliance with the Hardy-Weinberg equilibrium (HWB) conducted among groups of patients with RA and controls showed the presence of the proportion of A and G alleles equal to 0.73 and 0.27, as well as 0.7 and 0.3. In addition, the observed and expected frequencies of the A/A, A/G and G/G genotypes in the RA patient group corresponded to 0.56 and 0.53 (A/A), 0.35 and 0.39 (A/G), 0.09 and 0.07 (G/G) with insignificant difference ($\chi 2=1.33$; $p=0.242$; $df=1$); in the control group - 0.52 and 0.49 (A/A), 0.36 and 0.42 (A/G), 0.12 and 0.09 (G/G) also with no significant difference ($\chi 2=2.29$; $p=0.132$; $df=1$).

The established differences with the absence of statistical significance between the frequencies of genotypic variants of the A66G (rs1801394) polymorphism in the MTRR gene show compliance with the Hardy-Weinberg equilibrium ($p>0.05$).

The study of the nature of the distribution of the proportions of alleles and genotypes of the MTRR gene polymorphism (rs1801394) in the control group and patients with RA made it possible to determine their frequency for allele A - 70.2% and 29.8%, for G - 29.8% and 26.9%, respectively, in the studied group; for genotypes A/A - 52.3% and 55.7%, for genotype A/G - 35.8% and 34.9%, G/G - 9.4% and 11.9% corresponding to the groups (see Table 1).

The frequencies of major and minor alleles in subgroups of patients 1A and 1B were recorded in 71.6% and 76.6%, as well as in 28.4% and 23.4% of patients, respectively, in subgroups. At the same time, the difference was also felt in the proportion of genotypic variants of the MTRR gene polymorphism (rs1801394). So, if the major genotype A/A was registered in 56.8% and 53.1% of cases, the heterozygous genotype in 29.7% and 46.9% of cases, then the minor genotype, having maximum values among patients with articular form (13.5%),

was not registered in any case in patients with articular-visceral disease (see Table 1).

Table 1

Analysis of the frequency distribution of alleles and genotypes of gene polymorphism MTRR (rs1801394) among RA patients and controls

Group	Allele frequency				Frequency distribution of genotypes					
	A		G		A /A		A/G		G /G	
	n	%	n	%	n	%	n	%	n	%
General group of RA, (n = 106)	155	73.1	57	26.9	59	55.7	37	34.9	10	9.4
1A subgroup, (n=74)	106	71.6	42	28.4	42	56.8	22	29.7	10	13.5
1B subgroup, (n=32)	49	76.6	15	23.4	17	53.1	15	46.9	0	0.0
Control group, (n = 109)	153	70.2	65	29.8	57	52.3	39	35.8	13	11.9

The allele frequency of the MTRR gene polymorphism (rs1801394) in the general group of RA patients and in the compared group was characterized by no significant difference: allele A - 73.1% vs. 70.2% and allele G - 26.9% vs. 29.8% ($\chi2$=0.454; p=0.501; 0.866; 95% CI: 0.57-1.316) (see Table 2).

All three genotype variants did not differ significantly: A/A (55.7% versus 52.3%; $\chi2$=0.245; p=0.637; OR=1.145; 95% CI: 0.67-1.957); A/G (34.9% vs. 35.8%; $\chi2$=0.018; p=0.895; OR=0.962; 95% CI: 0.546-1.694); G/G (9.4% vs 11.9%; $\chi2$=0.350; p=0.568; OR=0.769; 95% CI: 0.322-1.836). The absence of statistically significant differences proves the absence of an association between the MTRR gene polymorphism (rs1801394) and the probable risk of developing RA in the general group of RA patients.

Table 2

Differences in the distribution of allele and genotype frequencies of the MTRR gene polymorphism (rs1801394) among RA patients and in the control group

Alleles and genotypes	Number of examined alleles and genotypes				χ^2	P	OR	95% CI
	General group		Control group					
	n	%	n	%				
A	155	73.1	153	70.2	0.454	0.501	1.155	0.76-1.756
G	57	26.9	65	29.8	0.454	0.501	0.866	0.57-1.316
A/A	59	55.7	57	52.3	0.245	0.637	1.145	0.67-1.957
A/G	37	34.9	39	35.8	0.018	0.895	0.962	0.546-1.694
G/G	10	9.4	13	11.9	0.350	0.568	0.769	0.322-1.836

The results of a comparative analysis of the frequency of variants of alleles and genotypes of the MTRR gene polymorphism (rs1801394) between patients with the articular form of RA and healthy individuals were also characterized by the absence of statistically significant differences in the distribution of

both alleles and genotypes for this polymorphic gene (see Table 3).

The difference between the G allele among patients of the 1A subgroup compared with the control was slightly less than one (28.4% versus 29.8%; χ2=0.088; p=0.772; OR=0.933; 95% CI: 0.59-1.475), between the A/A genotype - 1.2 (56.8% vs. 52.3%; χ2=0.354; p=0.566; OR=1.197; 95% CI: 0.662-2.164), between A/G genotype variants - less than one (29.7% vs. 35.8%; χ2=0.726; p =0.412; OR=0.759; 95% CI: 0.402-1.431) and G/G – 1.15 (13.5% versus 11.9%; χ2=0.101; p=0.756; OR=1.154; 95% CI: 0.477-2.792).

Table 3

Differences in the distribution of allele and genotype frequencies of the MTRR gene polymorphism (rs1801394) among RA subgroup 1A patients with articular form and in the control group

Alleles and genotyp	Number of examined alleles and genotypes				χ^2	P	OR	95% CI
	Subgroup 1A		Control group					
	n	%	n	%				
A	106	71.6	153	70.2	0.088	0.772	1.072	0.677-1.697
G	42	28.4	65	29.8	0.088	0.772	0.933	0.59-1.475
A/A	42	56.8	57	52.3	0.354	0.566	1.197	0.662-2.164
A/G	22	29.7	39	35.8	0.726	0.412	0.759	0.402-1.431
G/G	10	13.5	13	11.9	0.101	0.756	1.154	0.477-2.792

Analysis of the association of the rs1801394 polymorphism of the MTRR gene in the 1B subgroup of patients compared with the control showed that the minor G allele was distributed between patients and healthy individuals without significant difference (23.4% vs. 29.8%; χ2=0.991; P=0.327; OR=0.721; 95 % CI - 0.379-1.373). Very approximate values among those examined were found for the wild variant of the A/A genotype (53.1% versus 52.3%; χ2=0.007; P=0.937; OR=1.034; 95% CI: 0.472-2.263). At the same time, a trend towards an increase in the proportion of occurrence was revealed among patients of this subgroup in relation to the heterozygous A/G genotype, the frequency of which was 1.6 times higher compared to the control (46.9% vs. 35.8%; χ2=1.3; P=0.262; OR= 1.6; 95% CI: 0.716-3.504) (see Table 4)

Table 4

The difference in the distribution of frequencies of alleles and genotypes of the MTRR gene polymorphism (rs1801394) among patients of the 1B subgroup of RA with the articular-visceral form and in the control group

Alleles and genotyp	Number of examined alleles and genotypes				χ^2	P	OR	95% CI
	Subgroup 1B		Control group					
	n	%	n	%				
A	49	76.6	153	70.2	0.991	0.327	1.388	0.728-2.647
G	15	23,4	65	29.8	0.991	0.327	0.721	0.379-1.373
A/A	17	53.1	57	52.3	0.007	0.937	1.034	0.472-2.263
A/G	15	46.9	39	35.8	1.3	0.262	1.6	0.716-3.504
G/G	-	-	13	11.9	-	-	-	-

Conclusion. Thus, the results of the study on the study of the polymorphism of the MTRR gene (rs1801394) showed a correspondence between the differences between the expected and observed frequencies of the RHV genotype variants ($p>0.05$) both in the group of patients and in the comparison group. The proportion of occurrence of unfavorable allele (G) and genotypes (A/G and G/G) in the general group of patients with RA and among patients with the articular form of the disease does not significantly differ from those in the control ($\chi2<3.84$; $P>0.05$). Only among patients with the articular-visceral form, there is a tendency to increase the occurrence of the A/G genotype by 1.6 times ($\chi2=1.3$; $P=0.262$; OR=1.6; 95% CI: 0.716-3.504). Perhaps, with a larger coverage of the examined RA, a significant relationship with the development of the articular-visceral form of the disease could be established.

In this regard, the results of the study do not allow us to consider the MTRR gene polymorphism (rs1801394) as an independent genetic marker that increases the likelihood of developing RA.

References:

1. Alam J., Jantan I., Bukhari S.N.A. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. Biomed. Pharmacother. 2017, 92, 615–633.
2. Aletaha D., Neogi T., Silman A.J. et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580—8.
3. Arakawa Y, Watanabe M, Inoue N, et al. (2012) Association of polymorphisms in DNMT1, DNMT3A, DNMT3B, MTHFR and MTRR genes with global DNA methylation levels and prognosis of autoimmune thyroid disease. Clin Exp Immunol 170:194–201.
4. Chaabane, S., Messedi, M., Akrout, R., Ben Hamad, M., Turki, M., Marzouk, S., et al. (2018). Association of hyperhomocysteinemia with genetic variants in key enzymes of homocysteine metabolism and methotrexate toxicity in rheumatoid arthritis patients. Inflamm. Res. 67, 703–710. doi: 10.1007/s00011-018-1161-8.
5. Derksen V.F.A.M., Huizinga T.W.J., van der Woude D. The role of autoantibodies in the pathophysiology of rheumatoid arthritis. Semin. Immunopathol. 2017, 39, 437–446.
6. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care. 2012, 18(13 Suppl):S295–S302.
7. Gonzalez-Mercado, M. G., Rivas, F., Gallegos-Arreola, M. P., Moran-Moguel, M. C., Salazar-Paramo, M., Gonzalez-Lopez, L., et al. (2017). MTRR A66G, RFC1 G80A, and MTHFR C677T and A1298C polymorphisms and disease activity in mexicans with rheumatoid arthritis treated with methotrexate. Genet. Test Mol. Biomarkers 21, 698–704. doi: 10.1089/gtmb. 2017.0124.
8. Lv, S., Fan, H., Li, J., Yang, H., Huang, J., Shu, X., … Xiao, C. (2018). Genetic Polymorphisms of TYMS, MTHFR, ATIC, MTR, and MTRR Are Related to the Outcome of Methotrexate Therapy for Rheumatoid Arthritis in a Chinese Population. Frontiers in Pharmacology, 9. doi:10.3389/fphar.2018.01390.
9. Mirna Gisel Gonza´lez-Mercado et al. MTRR A66G, RFC1 G80A, and MTHFR C677T and A1298C Polymorphisms and Disease Activity in Mexicans with Rheumatoid Arthritis Treated with Methotrexate. // Genetic testing and molecular biomarkers. Volume 21, Number 11, 2017.Mary Ann Liebert, Inc. Pp. 1–7. DOI: 10.1089/gtmb.2017.0124.
10. Scherer, H. U., Häupl, T., & Burmester, G. R. (2020). The etiology of rheumatoid arthritis. Journal of Autoimmunity, 102400. doi:10.1016/j.jaut.2019.102400.