

## Influence of polymorphic variants of serotonin receptor 5-HTR2A T102C gene on mental status of patients with rheumatic arthritis depending on sex

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### Abstract

**Background:** In this study was determined the frequency of T102C polymorphism of the 5-HTR2A gene and its impact on the mental state of the patients with RA according to sex and gene variant polymorphism.

**Material and methods:** Molecular genotyping was performed using the technique of polymerase chain reaction. The degree of depression severity in the examined according to the Hamilton Anxiety Rating Scale was assessed. The levels of reactive and personal anxiety were defined according to the scale of Spielberger.

**Results:** In the study, we found that in women with RA was significantly less common homozygous CC as compared to male patients ( $p < 0.05$ ), and the clinical picture of patients with RA women with TT and TC genotypes there were significantly more frequent symptoms of fatigue, autonomic anxiety and depressive disorders (DS), high state, trait anxiety and symptoms of severe depression ( $p < 0.001$ ), whereas in women with CC genotype were found mild symptoms of DS. Our results showed that patients with RA men with genotype TC and TT, compared with women's characteristics, revealed mild to moderate severity of DS, and RA men patients with genotype CC did not demonstrate the symptoms of depression ( $p < 0.05$ ).

**Conclusions:** Depressive disorders with high levels of state and trait anxiety that affect significantly the course and manifestations in women with genotype TC and TT were detected in the vast majority of patients with RA.

**Key words:** rheumatic arthritis, polymorphism, serotonin receptor gene 5-HTR2A T102C.

### Introduction

Prolonged course of rheumatic arthritis (RA) leads to changes in life style and interests of patients, decrease of social and material status as well as the development of mental disorders [1]. It is demonstrated in the work of Y. K. Sikalo and N. A. Stanislavchuk [2] that patients with RA have complicated dynamics of mental disorders adversely influencing the course and prognosis of underlying disease. Depression and anxiety are the main manifestations of mental disorders. The most common conception of anxiety and depressive disorders is serotonin theory. Serotonin plays the major role in regulation of neuroendocrine rhythms, vascular tone, mood, sleep, appetite; it influences the behavior (including social), mood and emotions of a person [3]. Recent studies showed the disturbances in serotonergic system status to be associated with a number of psychopathologic phenomena [4]. The current knowledge of such mental disorders as depression and anxiety in RA patients demonstrates their significance in decrease of social adaptation of these patients, worsening of their social functioning. At the same time the relationship between mental disorders and clinical and social-psychological characteristics of RA patients is studied insufficiently [5].

The object of this work was to study the influence of polymorphic variants of serotonin receptor 5-HTR2A T102C gene on mental status of patients with rheumatic arthritis depending on sex.

### Material and methods

120 RA patients, 90 females (75.0%) and 30 males (25.0%) were studied. Their age ranged from 18 to 75 years, the mean age being  $51.5 \pm 12.04$  years. The distribution of genotype frequency of 2A serotonin receptor HTR2A T102C gene was determined in 100 patients taking a course of treatment at N. I. Pirogov Regional Clinical Hospital of Vinnitsa, Ukraine. Clinical characteristics of patients depending on genotype and sex are given in table 1.

Clinical diagnostics was made on the basis of ACR/EULAR criteria of 2010 [6]. Polymorphic sites of T102C in promotor regions of 5-HTR2A gene were determined by isolating genomic DNA with phenol-chloroform method using the kit for DNA/RNA isolation from blood serum or plasma (SPF "Li-Tech", Russia). Polymorphic sites of T102C gene were amplified with polymerase chain reaction.

Table 1

Clinical characteristics of RA patients, included in the study, depending on genotype and sex

Sign	RA patients (n=100)	TC (n=53)	TT (n=28)	CC (n=19)
Females, n (%)	73 (73.0)	42 (79.2)	21 (75.0)	10 (52.6)
Average age, females, years (M $\pm$ SD)	51.6 $\pm$ 12.0	49.5 $\pm$ 10.8	55.6 $\pm$ 13	51.7 $\pm$ 12.2
Males, n (%)	27(27.0)	11 (20.8)	7 (25.0)	9 (47.4)
Average age, males, years (M $\pm$ SD)	49.2 $\pm$ 11,8	51.7 $\pm$ 14.9	50.0 $\pm$ 5.0	45.6 $\pm$ 11.5
Seropositive RF, females, n (%)	45 (61.6)	19 (45.2)	17 (80.9)	9 (47.4)
Seropositive RF, males, n (%)	23 (85.2)	9 (81,8)	6 (85.7)	8 (88.8)

Examination of patients included: study of complaints, medical history and anamnesis vitae as well as the factors influencing psycho-emotional status of patients. Objective study of RA patients was done according to routine methods. Anxiety level was assessed by Spielberger State-Trait Anxiety Scale (STAI) [7]. Depressive status and depression severity were evaluated by Hamilton Depression Rating Scale (HDRS) [8]. Statistical data processing was done with Statistical package for Windows v. 8.0 using parametric and nonparametric methods. Modified Pearson's Chi-Squared criterion (p) was used to check the correspondence of empiric distribution of genotype frequency to theoretically expected Hardy-Weinberg equilibrium (steady distribution). Yates' continuity-corrected Shi-Squared test for small samples as well as two-tailed Fisher's exact test (F) was used in pair-wise comparison of allele and genotype frequencies in studied groups. Differences were considered significant if significance value was 95% (p<0.05).

**Results and discussion**

Study of genotype frequencies of polymorphic loci T102C of 5-HTR2A gene in RA patients depending on sex and according to Hardy-Weinberg distribution demonstrated heterozygous TC genotype to occur in 57.5% of females and 40.8% of males, homozygous TT genotype – almost with the same frequency in both sexes while homozygous CC genotype – more frequently in males than in females (p<0.01, respectively). The data are presented in table 2.

According to data received by Spielberger questionnaire among RA patients only 9 patients (7.5%) showed low level of state anxiety (SA) and 14 patients – low level of trait anxiety (TA) while moderate anxiety, both state and trait, was determined in 47 (39.2%) and 42 (35.0%) patients, respectively. The majority of patients (64 -53.3%) demonstrated high level of anxiety, both state and trait.

The analysis of SA and TA levels showed that irrespective of patients' genotype average indices of state 47.0[41.0;51.0] scores and trait 44.0[36;48] scores anxiety appeared to be higher in females than those in males – 38.0[32;45] and 38.0[34;44] scores, respectively – with no significant difference between them. High level of anxiety, both state and trait was observed significantly more frequently in females than in males: SA – 58.9% and 36.7% of patients ( $\chi_1^2=4.46, p=0.0346$ ); TA – 63.3% and 23.3% of patients ( $\chi^{2Y}=12.9; p=0.0003$ ), respectively.

The analysis of SA and TA indices in groups of patients with different variants of polymorphic sites of T102C gene de-

pending on sex showed that average values of state (p=0.0006) and trait (p=0.0135) anxiety in females with TC, were significantly higher than those in male patients with TC genotypes. At the same time in groups of male patients with TC and CC genotype there was a tendency to the increase of average value of SA in male patients with TC (p=0.085) in comparison with males CC. Besides in women with TC genotype there was significantly higher of SA values (p=0.0007) as compared to female patients with CC and TT (p=0.002) genotype. The data are presented in table 3.

**Table 3**

**Indices of state and trait anxiety in RA patients with different variants of polymorphic sites of T102C gene depending on sex Me [LQ;UQ]**

Genotype	Sex	TC	TT	CC
State anxiety (scores)	F	49.0 [46.0;54.0]**	42.0 [37.0;48.0]*	33.5 [30.0;42.0]**
	M	36.0 [32.0;45.0]	41.0 [38.0;45.0]	38.0 [30.0;45.0]
Trait anxiety (scores)	F	45.0 [36.0;48.0] #	44.0 [39.0;48.0]*	37.0 [32.0;42.0]*
	M	36.0 [34.0;39.0]	42.0 [34.0;48.0]	41.0 [37.0;44.0]

Notes: \* - confidence level p<0.01 between groups of female and/or male patients; # - confidence level p<0.05; \*\* confidence level p<0.001.

The data received in the course of study provided evidence that women with TC genotype had high SA levels significantly more frequently than moderate levels – 71.4±7.0% versus 28.6±6.9%, (p<0,001), and there were no cases of low anxiety levels among women with this genotype. Among male patients with TC genotype there was a tendency to the increase of the number of patients with high SA level (p=0.092) as compared to those with low SA level. There were no significant differences in this index between RA patients, both women and men, with TC genotype. Among women with TT genotype moderate and high SA levels occurred with similar frequency -42.9% and 57.1% (p>0.05), respectively, and there were no patients with low anxiety level. At the same time 71.4% of men with TT genotype showed the signs of moderately evident SA and the tendency (p=0.052) to decreased number of patients with high and low SA levels by contrast to patients having moderate anxiety levels which occurred with similar frequency

**Table 2**

**Genotype frequency of polymorphic loci T102C of 5-HTR2A gene in RA patients depending on sex (%)**

Locus	Sex	n	Genotypes			Alleles	
			TC	TT	CC	T	C
T102C	M	27	40.8 %	25.9 %	33.3%	53.7 %	46.3%
	$\chi^2 = 0.88; p = 0.348; \text{Fisher's exact test: } p = 0.348   F(ST) = 0.330$						
	F	73	57.5%	28.8%	13.7%	57.5%	42.5%
	$\chi^2 = 2.3; p = 0.129; \text{Fisher's exact test: } p = 0.125   F(ST) = 0.411$						

in 14.3%, respectively. Significant increase of the number of female patients with high anxiety level in comparison with male patients was established between female and male groups with TT genotype - 57.1% and 14.3% ( $p < 0.05$ ), respectively.

High SA level was determined in 50.0% of women and 33.3% of men with CC genotype while low and moderate levels - in 20.0% and 30.0% of women and 22.2% and 44.4% of men, respectively. No significant difference of this index between the groups of female and male patients with CC genotype was detected.

High TA level was diagnosed in women with TC genotype significantly more often than low one - 52.4% versus 16.7% ( $p < 0.001$ ) and moderate - 52.4% versus 30.9% ( $p < 0.05$ ), respectively, while 54.5% of men with TC genotype had low TA level, 27.3% and 18.2% - high and moderate levels, respectively. The tendency ( $p = 0.092$ ) to the increase of number of patients with low TA level was observed between the groups of patients with high and moderate TA levels.

No patients had low TA level both among women and men with TT genotype while in women with TT genotype high TA level was seen significantly more often than moderate one - 85.7% versus 14.3% ( $p < 0.001$ ), and in men moderate TA level was detected significantly more often than high one - 71.4% versus 28.6% ( $p > 0.05$ ). Between the groups of female and male patients there was significant increase of male patients with TT genotype and moderate TA level in comparison with female patients ( $p < 0.01$ ) as well as significant increase of female patients with high TA level by contrast to men ( $p < 0.01$ ).

Among female patients with genotype CC high TA level was detected in 50.0%, moderate - in 40.0% and low - in 10.0% of women. There was a tendency to the increase of the number of women with high TA level ( $p = 0.062$ ) as compared to patients with low TA level. Among men with CC genotype there were no patients with low TA level while patients with moderate TA level occurred significantly more often than those with high TA level - 88.9% versus 11.1% ( $p < 0.01$ ). Moderately evident TA was detected significantly more often in men with CC genotype than in women - 88.9% versus 40.0% ( $p < 0.05$ ) while women showed the tendency to the increase of the number of patients with high TA level by contrast to men ( $p = 0.086$ ), respectively.

The results obtained showed depressive disorders (DD) of various degree of severity in 105 (87.5%) RA patients by Hamilton depression scale. According to this scale 55 patients (45.8%) had mild manifestations of DD, 33 patients (27.5%) - moderately severe, 11 patients (9.2%) - severe and 6 patients (5.0%) - extremely severe signs. Incidence of DD

in patients with RA depending on sex was determined, it being significantly higher in women than in men - 83 (92.2%) versus 22 (73.3%).

Average values of depression by Hamilton scale in women and men ill with RA with TT and CC genotypes appeared to be nearly identical without any significant differences in the values ( $p > 0.05$ ). The corresponding data are given in table 4.

The analysis of depressive disorders indices in groups of patients with different variants of polymorphic sites of T102C gene depending on sex showed that average values of depression by Hamilton scale ( $p = 0.0001$ ) in females with TC, were significantly higher than those in male patients with TC genotypes. At the same time in the group of female patients with CC genotype there was significant decrease of DD as compared to female patients with TT ( $p = 0.02$ ) and TC ( $p = 0.015$ ) genotype. The analysis of depression by Hamilton scale indices in groups of patients with different variants of polymorphic sites of T102C gene depending on sex showed that average values DD in male patients with TT genotype ( $p = 0.0036$  and  $p = 0.0004$ , respectively), were significantly higher than those in male patients with TC and CC genotypes.

In female patients with TT genotype moderately severe DD occurred significantly more often ( $42.9 \pm 10.8\%$ ) than in patients with no signs of depression ( $4.8 \pm 4.7\%$ ,  $p < 0.01$ ), patients with signs of severe depression ( $14.2 \pm 7.6\%$ ,  $p < 0.05$ ) and extremely severe depression ( $9.5 \pm 6.4\%$ ,  $p < 0.05$ ). There was no significant difference in values between groups of female and male patients with TT genotype. No signs of extremely severe DD were found among men with TT genotype.

In female patients with CC genotype mild DD occurred significantly more often ( $70.0 \pm 14.5\%$ ) than in patients with no signs of depression, patients with signs of mild and severe depression having similar frequency -  $10.0 \pm 9.5\%$  ( $p < 0.05$ ). In 66.7% of RA male patients with CC genotype signs of mild depression occurred and 33.3% of patients had no signs of depression. There was no significant difference in values between groups of female and male patients with CC genotype. No signs of extremely severe DD were found among men and women with CC genotype. Besides, no signs of mild and moderately severe DD were detected in men with CC genotype.

**Conclusions**

The study found out that RA female patients with TC genotypes had high levels of state and trait anxiety indicating evident psychoemotional stress with development of persistent anxiety as a trait of character. The presence of psychoemotional stress in female and male patients with CC genotype

**Table 4**

**Indices of depressive disorders in RA patients with different variants of polymorphic sites of T102C gene depending on sex Me [LQ;UQ].**

TC genotype		TT genotype		CC genotype	
F	M	F	M	F	M
14.0 [11.0;17.0]	8.0 [6.0;8.0]**	15.0 [12.0;18.0]	16.0 [12.0;18.0]	10.0 [8.0;13.0]	9.0 [7.0;12.0]*

Notes: \* - confidence level  $p < 0.001$  between groups of female and/or male patients.

was suggested by signs of moderately severe anxiety were detected. Moderately severe and severe depressive disorders were common in women with TT genotype while the majority of women with TC and CC genotypes as well as men with CC genotype had mild depressive disorders.

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