The cytokine of the tumor necrosis factor (TNF) family B-cell activating factor (BAFF) (TNFSF13B) that regulates the proliferation, differentiation, and survival of B cells is assumed to be involved in the pathogenesis of multiple sclerosis (MS).

Objective: to analyze the association of BAFF gene polymorphisms (rs1224141, rs9514827) with the progression rate and frequency of MS exacerbations.

Patients and methods. A total of 100 Caucasian patients (24 males and 76 females) with relapsing-remitting MS, who were born and lived in the Altai Territory of the Russian Federation, were examined. Genotyping was performed by real-time polymerase chain reaction using competitive TaqMan probes.

Results and discussion. The annual risk of a >0.75 point disability increase in the Expanded Disability Status Scale (EDSS) score was ascertained to be associated with the first remission duration of less than 2 years, with the C/G genotype of BAFF (rs1224141) in males and females, and with the C/C genotype of BAFF (rs9514827) in females. The likelihood of the first remission duration of less than 2 years was increased in female carriers of the G allele of BAFF (rs1224141). There was no association of BAFF gene polymorphisms (rs1224141, rs9514827) with the frequency of MS exacerbations.

It seems promising to further study the role of BAFF in the pathogenesis of MS and the effect of this cytokine on the specific features of the course of the disease. The investigation results will be able to predict the efficiency of MS therapy with anti-BAFF drugs and to identify criteria for their individualized use.

Conclusion. In patients with MS in the Altai Territory of the Russian Federation, the risk for a high MS progression rate is related to the carriage of BAFF genotypes with rare alleles in homozygous state: G/G polymorphism rs1224141, C/C polymorphism rs9514827 in combination with the female sex. The G allele of BAFF (rs1224141) in women is associated with the risk of the unfavorable prognostic duration of the first MS remission of less than 24 months.

Keywords: multiple sclerosis; B-cell activation factor; TNFSF13B; polymorphisms

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For reference: Smagina IV, Elchaninova SA, Palashchenko AS. Association of BAFF gene polymorphisms with multiple sclerosis progression. Neurology, Neuropsychiatry, Psychosomatics. 2020;12(1):22–26.

DOl: 10.14412/2074-2711-2020-1-22-26
Disability was assessed on the Expanded Disability Status Scale (EDSS) [12]. The frequency of exacerbations was calculated as the number of exacerbations per year, the rate of progression (PR) of MS was calculated as the ratio of the EDSS points at the time of examination to the duration of the disease [13].

All patients included in the study were Caucasians by phenotypic characteristics, were born and lived in the Altai region of Russia. Inclusion of patients in the study was carried out by random numbers from the population of MS patients of the Altai region.

The inclusion criteria were: absence of disease-modifying treatments; disability ≤6.5 EDSS points. This criterion is determined taking into account that at disability >6 EDSS points patients have irreversible persistent neurological disorders, which, as a rule, persist for several years until the death of the patient. For such cases, the calculated low PR MS does not reflect the favorable course of the disease.

To analyze the association of PR MS with gene polymorphisms, patients were divided into three subgroups: low PR MS (<0.25 EDSS points/year) — 26 (26%) patients, middle PR MS (0.25 to 0.75 EDSS points/year) — 55 (55%) patients and high PR MS (>0.75 EDSS points/year) — 19 (19%) patients.

The relationship of genetic features with the frequency of exacerbations was evaluated in a 3-year prospective follow-up of patients with neurological disorders—according to the results of retrospective analysis. The characteristics of patients are presented in Table 1.

In the study of genomic polymorphisms, DNA was isolated from venous blood using a standard procedure involving separation and lysis of blood cells, followed by hydrolysis of proteins by protease K, purification of DNA with a mixture of phenol-chloroform with ethanol precipitation. Genotyping was performed by real-time polymerase chain reaction using competing TaqMan probes complementary to polymorphic DNA sites.

Statistical analysis was performed in Statistica (StatSoft Statistica 10.0.1011.0 Russian Portable, StatSoft, Inc., USA). The Mann-Whitney test was used to compare groups. The odds ratio (OR) was calculated by logistic regression analysis. The accordance of the genotype distribution to the Hardy-Weinberg equilibrium was evaluated by the chi-square test using the DeFinetti program on the website of the Institute of Human Genetics (Munich, Germany; https://ihg.helmholtz-muenchen.de/cgi-bin/hlw/hlw1.pl). Significance level p<0.05 was accepted for all statistics.

The study was approved by the Ethics Committee of the Altai state medical University (Barnaul, Russia). All patients signed informed consent to participate in the study.

Results. Analysis of the BAFF gene single nucleotide polymorphisms (rs1224141, rs9514827) showed that the distribution of genotypes corresponds to the Hardy-Weinberg distribution (p=0.84 and p=0.79, respectively). The alleles and genotypes distributions of the BAFF single gene specificity are presented in Table 2.

As identify by the evaluation of the relationship of MS progression with the analyzed of the BAFF gene single-nucleotide polymorphisms, the G/G genotype in BAFF (rs1224141) is associated with high PR MS. Associations of alleles or genotypes with low or medium PR MS were not revealed (Table 3).

Previously, the relationship of PR MS with gender was revealed in the Altai region [14]. It turned out that the PR MS for men is 2 times higher than for women. A greater risk of high PR MS was found in men [14]. In this regard, the analysis of associations of male or female combinations with genotypes and alleles of the BAFF gene polymorphisms was carried out. The association of female sex and C/C genotype in BAFF (rs9514827) with an increased risk of high PR MS was found (Table 4).

Taking into account the high prevalence of adverse course of MS in patients with a late age of disease onset and a short period of first remission [14] the analysis of the relationship of these features of the course of MS with BAFF polymorphisms (rs1224141, rs9514827) was performed. The relationship between the features of clinical manifestations of the disease debut and polymorphisms of the BAFF gene was not found. It was found that the duration of the first remission less than 24 months in the study participants is associated with an increased risk of high PR MS (OR 4.32; CI 0.88—21.17; p=0.045). However, the duration of the first remission less than 24 months was associated with the carrier of the G allele in BAFF (rs1224141) (OR 7.06; CI 1.02—48.70; p=0.040) only in women. The relationship of the frequency of exacerbation of MS with the carrier of genotypes and alleles of the BAFF polymorphisms was not found (Table 5).
Discussion. It is accepted that the pathogenesis of autoimmune diseases, including MS, involves cytokines of the TNF family. These cytokines affect the beginning of immune inflammation, demyelination, and apoptosis of oligodendrocytes in MS [15]. Thus, TNF-α is one of the most powerful proinflammatory cytokines, Fas-ligand causes apoptosis of target cells in the central nervous system, and CD40-ligand provides a stimulating signal for intercellular interaction of T- and B-cells [15].

For many years, MS was seen as a disease mediated primarily by T-cells. In recent decades, an important role of B cells has been established in the development and progression of MS through antigen presentation and production of multiple cytokines [6, 16, 17]. In this regard, more and more attention is paid to the study of the laws of functioning of the B-cell immunity in MS in connection not only with antibody production, but also with the participation in antigen presentation and production of many cytokines. The cytokine of the TNF-BAFF family is one of the regulators of B-cell proliferation, differentiation and survival [5].

Table 3. Relative risk of high rate of multiple sclerosis progression depending on BAFF genotypes (rs1224141, rs9514827)

| Genotype | MS patients, % | Odd Ratio Mean (95% CI) | P-value |
|----------|----------------|-------------------------|---------|
|          | low and moderate rate of multiple sclerosis progression | high rate of multiple sclerosis progression | |
| BAFF (rs1224141) | | | |
| T/T | 93 | 7 | 0.95 (0.28-3.26) | 0.932 |
| T/G | 95 | 5 | 0.55 (0.15-2.03) | 0.370 |
| G/G | 98 | 2 | 15.40 (1.23-192.17) | 0.031 |
| BAFF (rs951427) | | | |
| T/T | 95 | 5 | 0.83 (0.25-2.80) | 0.773 |
| T/C | 95 | 5 | 0.69 (0.20-2.31) | 0.544 |
| C/C | 97 | 3 | 2.85 (0.64-12.78) | 0.173 |

Table 4. Relative risk of high rate of progression of multiple sclerosis depending on gender and rare genotypes and alleles of BAFF (rs1224141, rs9514827)

| Genotype (Allele) | Odd Ratio Mean (95% CI) | P-value |
|-------------------|-------------------------|---------|
| BAFF (rs1224141) | | |
| G/G, female | 10.0 (0.52-191.33) | 0.119 |
| G, female | 0.74 (0.04-12.62) | 0.832 |
| G/G, male | N/A | N/A |
| G, male | 9.17 (0.22-378.52) | 0.213 |
| BAFF (rs9514827) | | |
| C/C, female | 5.9 (1.09-31.90) | 0.036 |
| C, female | 4.13 (0.47-36.08) | 0.191 |
| C/C, male | N/A | N/A |
| C, male | 0.64 (0.02-19.48) | 0.788 |

Table 5. Relative risk of exacerbation of multiple sclerosis more often than one per year depending on the alleles and BAFF genotypes (rs1224141, rs9514827)

| Genotype (Allele) | Odd Ratio Mean (95% CI) | P-value |
|-------------------|-------------------------|---------|
| BAFF (rs1224141) | | |
| T/T | 1.77 (0.75-4.20) | 0.192 |
| T/G | 0.71 (0.30-1.67) | 0.433 |
| G/G | N/A | N/A |
| T | 3.68 (0.75-18.01) | 0.100 |
| G | 0.27 (0.06-1.33) | 0.102 |
| BAFF (rs9514827) | | |
| T/T | 1.59 (0.69-3.63) | 0.273 |
| T/C | 0.71 (0.31-1.61) | 0.414 |
| C/C | 0.76 (0.20-2.83) | 0.672 |
| T | 1.91 (0.54-6.69) | 0.312 |
| C | 0.5 (0.15-1.84) | 0.311 |

Note: NA (not available) in this table and table 5 is not determined due to the low frequency of genotype.
Based on this fact, we analyzed the association of the BAFF gene polymorphisms (rs1224141, rs9514827) with PR MS and the frequency of exacerbations of in MS a group of Caucasians living in the Altai region of the Russia.

It was found that the increased risk of high PR remitting MS is associated with the duration of the first remission of less than 2 years, the G/G genotype in BAFF (rs1224141), in women also with the C/C genotype in BAFF (rs9514827). The probability of the duration of the first remission of the disease is less than 24 months increased in women-carriers of the allele G in BAFF polymorphism (rs1224141), rs9514827) with the frequency of exacerbations of MS was not revealed.

It should be noted that currently, taking into account the pathogenetic significance of BAFF and its homologue APRIL (A proliferation-inducing ligand), several drugs that inhibit the effects of these cytokines have been produced and applied in practice in a number of autoimmune diseases: anti-BAFF humanized monoclonal antibodies (Belimumab and LY2127399), a soluble TACI receptor that binds BAFF cytokines and APRIL (soluble decoy TACI-Fc fusion protein, Atacicept), etc. [6].

Further study of the role of BAFF in the pathogenesis of MS, the influence of this cytokine on the features of the disease course is promising. The results of the research will allow to predict the effectiveness of MS therapy with anti-BAFF drugs, to determine the criteria for their individualized use.

Conclusion. In patients with MS in the Altai region of the Russia, the risk of high PR MS is associated with homozygosity of rare alleles of BAFF gene polymorphism: G/G BAFF (rs1224141), C/C BAFF (rs9514827) in combination with the female sex. Allele G BAFF (rs1224141) in women is associated with the risk of prognostically unfavorable duration of the first remission of MS less than 24 months.

Acknowledgments The authors express their gratitude to the specialists of the pharmacogenomics group of the Institute of Chemical Biology and Fundamental Medicine SB RAS (Novosibirsk, Russia) M. L. Filippenko and E. N. Voronina for their assistance in genotyping.

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**Received** July 17, 2019

**Declaration about financial and relationships.**
This is a non-funded investigation. The authors are fully responsible for submitting the final version of the manuscript for publication. The final version of the manuscript has been approved by all co-authors.