Polymorphic variants in the *PVT1* locus affect multiple sclerosis severity

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Multiple sclerosis (MS) is a chronic autoimmune disease, in the pathogenesis of which the concurrence of demyelination of central nervous system (CNS) axons and neurodegeneration plays a role and which is accompanied by progressive neurological dysfunction. Long-term monitoring of patients with MS is needed to rate its severity according to existing scales; it is therefore very relevant to search for genomic markers that can predict the rate of disease progression at early stages. The impact of polymorphic variants in the PVT1 locus on MS severity has not been previously studied.

Objective: *to analyze the association of the polymorphic variants rs4645948 in the MYC gene and rs2114358 and rs4410871) in the PVT1 genes with MS severity according to the Multiple Sclerosis Severity Scale (MSSS) separately and as part of biallelic combinations, as well as the possible linkage disequilibrium of the studied single nucleotide polymorphisms for establishing the independence of the observed associations.*

Patients and methods. *The investigation enrolled 468 Russian MS patients who did not take immunomodulating drugs before blood testing. The patients were divided into two groups: 1) relatively mild MS (MSSS ⩽3.5) and 2) relatively severe MS (MSSS >3.5). The polymorphic variants in the PVT1 locus were genotyped by a real-time polymerase chain reaction assay.*

Results and discussion. *In the MS study group, the carriage of the allele of PVT1 (rs2114358)*G turned out to be associated with the severe course of the disease (pf=0.042; odds ratio (OR)=1.41). The significance of the association increases in the simultaneous carriage of this allele with another variant of the same gene – PVT1 (rs4410871)*T (pf=0.024; OR=1.58). There was no linkage disequilibrium between the components of the biallelic combination.*

Conclusion. *The polymorphic variants in the PVT1 locus are associated with the severity of MS.*

Keywords: *multiple sclerosis; Multiple Sclerosis Severity Scale; genetic polymorphism; association analysis; multilocus analysis.*

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For reference: *Kiselev IS, Kozin MS, Baulina NM, et al. Polymorphic variants in the PVT1 locus affect multiple sclerosis severity. Nevrologiya, neiro-psikhiatriya, psikhosomatika = Neurology, Neuropsychiatry, Psychosomatics. 2020;12(Suppl. 1): 4–8.* ***DOI:*** *10.14412/2074-2711-2020-1S-4-8*

Multiple sclerosis – (MS) is a chronic disease of the autoimmune nature, in the pathogenesis of which a combination of axon demyelination in the central nervous system and neurodegeneration plays a role, and which is accompanied by progressive neurological dysfunction [1]. The steady increase in neurological deficit leads to irreversible disability of patients at a young age, which determines the important social and economic significance of the disease. MS occurs almost all over the globe, but its prevalence in different populations varies greatly. In the Russian Federation, it varies from 30 to 70 cases per 100 thousand population [2].

The course of MS is characterized by pronounced clinical heterogeneity. The main types of MS: remitting (RMS), characterized by alternating periods of exacerbation and remission; secondary-progressive, developing in patients with RMS in the absence of effective therapy and accompanied by a steady deterioration of neurological symptoms; primary-progressive, in which the increase in neurological disorders is observed from the very beginning of the disease. At the same time, even in patients with the most common type of MS – RMS – the age and symptoms at the onset, frequency and severity of exacerbations, duration of remissions, etc., differ significantly, which makes it seriously difficult to predict the rate of the disease progression [3].

The most common tool for determining the rate of MS progression in a particular patient is the MS severity scale (Multiple Sclerosis Severity Scale, MSSS), which is based on the assessment of the patient's increasing degree of disability over time, defined by the patient's score on the Expanded Disability Status Scale (EDSS) [4]. One of the major drawbacks of MSSS is the need for long-term monitoring of patients for at least a year, so the search for markers of MS severity that would allow predicting the rate of its progression in the early stages is highly relevant.

MS is a complex polygenic disease; both the risk of its development and clinical characteristics can be largely determined by the genetic characteristics of a particular patient [5]. The most informative method for studying the genetic architecture of polygenic diseases is the genome wide association study (GWAS), but to achieve a full-genome level of significance, such studies must include extensive study groups. For example, the latest work, which shows the association between MS and 233 single nucleotide polymorphisms (SNPs) across the entire genome, includes more than 100 thousand MS patients and healthy individuals as controls [6].

When searching for polymorphic variants associated with the severity of MS, much stricter requirements for homogeneity of groups by clinical characteristics and treatment status are used than when studying predisposition to the disease. As a result, studies of this type using the GWAS method include limited groups of patients (usually no more than 5–10 thousand) and have not yet given significant results at the genome-wide level [7].

At the same time, the association of various polymorphic variants of the genome with the severity of MS has already been shown in the studies using the "candidate gene" approach, which does not require large samples [8, 9].

The long non-coding RNA (ncRNA) *PVT1* gene is a candidate gene that is promising for searching for polymorphic variants associated with the severity of MS. Previously, we obtained data on the association of SNP rs2114358 of the *PVT1* gene with the risk of developing MS [10]. Another polymorphic variant, rs4410871, was identified as associated with MS in GWAS [6]. Recent data obtained in experiments on animals and on the cell line of myoblasts indicate that ncRNA *PVT1* may be involved in the regulation of mitochondria [11], whose dysfunction is crucial to the process of neurodegeneration in MS [1]. The association of polymorphisms of the *PVT1* locus with MS can also be explained by the contribution to the development of the disease of the proto-oncogene *MYC*, which is located in the immediate vicinity of *PVT1* on chromosome 8. The protein product of the *MYC* gene, like *PVT1*, is involved in the regulation of mitochondria and, in addition, takes part in the regulation of the cell cycle, apoptosis and cell metabolism. The Myc protein is able to stimulate inflammatory responses in MS [12], while the expression level of its gene depends on the alleles and genotypes of the rs4645948 polymorphic variant located in the 5'-untranslated region of the *MYC* gene [13]. Thus, from the available data, it can be concluded that the *PVT1* and *MYC* genes are involved in the pathogenesis of MS, which means that their functional polymorphisms can participate in determining the rate of the disease progression.

The aim of the study was to genotype and then analyze the association of polymorphic variants of the *MYC* (rs4645948) and *PVT1* (rs2114358, rs4410871) genes with the severity of MS on the MSSS scale, both individually and as part of biallelic combinations; to analyze the possible linkage disequilibrium of the studied SNPs to establish the independence of the observed associations.

Patients and methods. The study included 565 unrelated patients with MS (394 women and 171 men, the mean age at the time of blood sampling was 38.7±10.7, the age of MS debut 27.4±9.2 years), who were observed in the Moscow Center for Multiple Sclerosis or in the Moscow Inter-District Department of Multiple Sclerosis in City Clinical Hospital No. 24 in Moscow. MS was diagnosed according to the McDonald Criteria (2010) [14]. Patients who started taking immunomodulatory drugs within the first year after the diagnosis or who had minimal neurological symptoms during the follow-up period (EDSS ⩽1 at the end point of follow-up) were excluded from the analysis. The final group of MS patients consisted of 468 subjects whose clinical characteristics are presented in Table 1. The severity of MS was assessed for each patient on the MSSS scale [4] based on the EDSS indicator and the duration of the disease. For associative analysis, the patients were divided into two groups. The 1st group included 218 patients with relatively mild MS (MSSS ⩽3.5), and the 2nd group included 250 patients with relatively severe MS (MSSS >3.5). All MS patients were ethnic Russians (according to the survey). All of them signed the informed consent to participate in the study. The study was approved by the Ethics Committee of the Pirogov Russian National Research Medical University of the Ministry of Health of Russia.

Table 1. Clinical characteristics of MS patients

Parameters	Value
Females/Males	2.5/1
MS duration, years	8.9±6.6
Age at MS onset, years	27.6±9.2
EDSS at baseline	2.6±1.2
MSSS at baseline	3.9±1.9

DNA was isolated from whole blood using commercial QIAamp DNA Blood MidiKit (Qiagen, Germany). Genotyping of polymorphic variants of the *MYC* (rs4645948) and *PVT1* (rs2114358, rs4410871) genes was performed by real-time polymerase chain reaction on StepOnePlus detecting amplifiers (Thermo Fisher Scientific, USA) using commercial TaqMan Predesigned SNP Genotyping Assay Kit (Thermo Fisher Scientific, USA).

The analysis of the association of carriage of alleles and genotypes of the studied SNPs and their combinations with the severity of MS on the MSSS scale was performed using the APSampler software (https://sourceforge.net/projects/apsampler/). The strength of associations was expressed as the odds ratio (OR). The associations for which OR 95% confidence interval (CI) did not cross 1, were considered significant, and the value of p, according to the exact Fischer criterion, was >0.05. To assess the nature of interaction (epistatic or additive) between the components of the identified combination, we calculated p values in

Table 2. The frequency of carriage of alleles and genotypes of the studied polymorphic variants in patients with severe and mild MS course

Variants	Allele/ genotype	Number of carriers, n (%)		p_f	OR [95% CI]*
		MSSS≤3.5 (n=218)	MSSS>3.5 (n=250)		
MYC rs4645948	C	214 (100.0)	244 (99.6)	n.s.	–
	T	12 (5.6)	6 (2.4)	n.s.	–
	C/C	202 (94.4)	239 (97.6)	n.s.	–
	C/T	12 (5.6)	5 (2.0)	0.038	0.35 [0.12–1.01]
	T/T	0 (0.0)	1 (0.4)	n.s.	–
PVT1 rs2114358	A	186 (85.3)	211 (84.7)	n.s.	–
	G	122 (56.0)	160 (64.3)	0.042	1.41 [1.01–2.05]
	A/A	96 (44.0)	89 (35.7)	0.042	0.71 [0.49–0.99]
	A/G	90 (41.3)	122 (49.0)	n.s.	–
	G/G	32 (14.7)	38 (15.3)	n.s.	–
PVT1 rs4410871	C	204 (93.6)	235 (94.0)	n.s.	–
	T	93 (42.7)	112 (44.8)	n.s.	–
	C/C	125 (57.3)	138 (55.2)	n.s.	–
	C/T	79 (36.2)	97 (38.8)	n.s.	–
	T/T	14 (6.4)	15 (6.0)	n.s.	–

Notes: n.s.– not significant; * for significant p_f.

the exact three-way Fisher-like interaction numeric test (FLINT) and synergy factor (SF) included in the APSampler tools. The interaction was considered epistatic if the pflint value was <0.05, and 95% CI for SF did not cross 1. The analysis of linkage disequilibrium of the studied SNPs was performed using the HaploView algorithm (https://www.broadinstitute.org/haploview/haploview). To determine the linkage force, we used the r^2 metric and the normalized Lewontin linkage disequilibrium coefficient (D'). At D'<0.7 and r^2<0.5, the linkage between polymorphic variants was regarded as weak, at D'>0.7 and r^2>0.5 – as significant, and at D'=1 and r^2>0.8 – as strong.

Results. The frequency of carrying alleles and genotypes of SNP from the *PVT1* locus in the groups of MS patients with relatively mild (MSSS ⩽3.5) and relatively severe (MSSS >3.5) (Table 2) disease course was determined.

As can be seen from Table 2, the carriage of the allele *PVT1* (rs2114358)*G is associated with severe MS (pf=0.042; OR=1.41; 95% CI 1.01–2.05), while the genotype *PVT1* (rs2114358)*A/A is significantly more common in patients with mild disease (pf=0.042; OR=0.71; 95% CI 0.49–0.99). In patients with mild MS, the frequency of carrying the *MYC* (rs4645948)*S / T genotype is also increased (pf=0.038; OR=0.35). However, 95% DI for OR crosses 1, so this association was not classified as significant. For SNP *PVT1* (rs4410871), no significant associations were found with the severity of MS assessed on the MSSS scale.

To search for allelic combinations that can act as markers of the severity of MS, a multi-locus analysis was performed. We identified allelic combination – *PVT1* (rs2114358)*G + *PVT1* (rs4410871)*T – the carriage of which is associated with severe MS (pf=0.024; OR=1.58; 95% CI 1.03–2.43). We also analyzed the nature of interactions between the components included in the combination. The values of pflint and SF were insignificant, which indicates the additive nature of the interaction.

We studied the possible linkage disequilibrium between the polymorphic variants of the *MYC* (rs4645948) and *PVT1* (rs2114358, rs4410871) genes to establish its role in the observed associations (see Fig.1). In the studied group of MS patients, the values of D' and r^2 did not exceed 0.44 and 0.003, respectively, which indicates the absence of a pronounced linkage between the polymorphic variants. The calculation of the D' and r^2 metrics based on the data from the project *1000 Genomes* also shows that there is linkage disequilibrium between the studied variants: the values of D' and r^2 for the studied SNPs in European populations do not exceed 0.674 and 0.004, respectively.

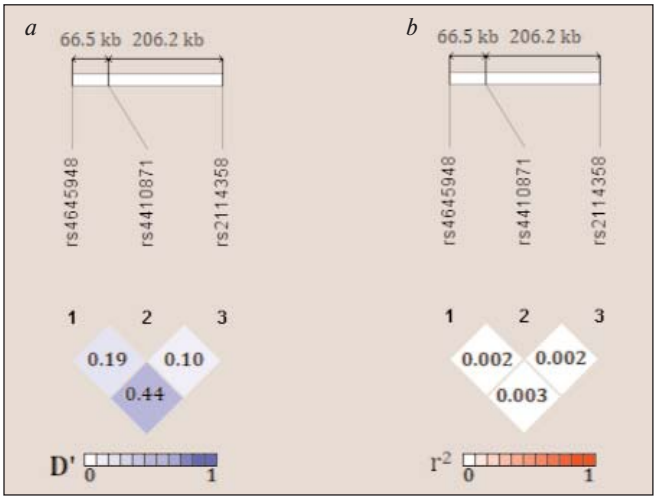

Figure 1. *Results of analysis of linkage disequilibrium between the polymorphic variants in the MYC (rs4645948) and PVT1 (rs2114358, rs4410871) genes in the studied group of MS patients. The color gradient denotes the coupling force expressed in the values D' (a) and r2 (b)*

Discussion. This study shows for the first time that the carriage of the *PVT1* allele (rs2114358)*G is a marker of severe MS on the MSSS scale in Russian patients, while the alternative genotype *PVT1* (rs2114358)*A/A is more common in patients with a mild course of the disease.

The polymorphic variant rs2114358 is located in the fifth intron of the long non-coding RNA *PVT1* gene. RNA molecules of this class are widely involved in regulating the differentiation and functioning of immune cells under normal and pathological conditions. Thus, high levels of PVT1 are normally found in monocytes, CD4+ and CD8+ T-lymphocytes, and its pathological increase is observed in patients with acute and chronic leukemia, multiple myeloma, and a number of other malignancies [15, 16]. In recent years, data have appeared on the association of PVT1 levels with various autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis [17, 18].

MIR1204, MIR1205, MIR1206 and *MIR1207* genes located in the *PVT1* gene can also contribute to the functioning of the immune system in normal and pathological conditions. They encode microRNAs – small (20–25 nucleotides) single-stranded non-coding RNA molecules that can complementarily or partially complementarily bind to the target mRNA and mediate its degradation or inhibition of protein product synthesis. The polymorphic version of rs2114358 is located in the pre-miR-1206 region, which means that it can affect the efficiency of its splicing. The biological functions of miR-1206 are still poorly understood and, we hope, they will be identified in the course of further research.

Although several polymorphic variants of the *PVT1* locus are among the GWAS-identified markers of MS risk, they do not include SNP rs2114358 [6]. Indeed, when analyzing its association separately with the two most common types of MS – remitting and primary-progressive – we did not get significant results [19]. However, the use of multi-locus analysis allowed to increase the statistical power of the study and reveal the association of the rs2114358*G allele with a high risk of developing RMS when compared with both healthy volunteers [10] and patients with primary-progressive MS [20] as part of allelic combinations.

We did not observe a significant association between SNP *PVT1* (rs4410871) located in the first exon of the *PVT1* gene and the severity of MS on the MSSS scale. However, if the *PVT1* allele (rs4410871)*T is part of a biallelic combination with the variant of the same *PVT1* gene (rs2114358)*G which is independently associated with the severity of MS, the level of significance and OR for this combination is higher than for *PVT1* (rs2114358)*G alone. Moreover, the absence of linkage disequilibrium between these two polymorphisms indicates the independence of their effects. There is no epistatic interaction between them either. Thus, when using multi-locus analysis, we found a connection of the *PVT1* allele (rs4410871)*T with the severity of MS course. At the same time, in contrast to SNP rs2114358, the polymorphic variant rs4410871 is an independent marker of MS risk identified in GWAS [6].

The results of this study complement the data previously obtained, and indicate the connection of two polymorphic variants of the gene *PVT1* (rs2114358 and rs4410871) with the risk of MS development and its severity; the only difference is that without the use of multi-locus sequence analysis, the significance level of the association with risk of development of MS was sufficient for rs4410871, and with the severity of its course – for rs2114358.

Although we observed a tendency towards association between the heterozygous genotype rs4645948*C/T and a mild course of MS, it is obvious that the size of the study sample was insufficient to obtain a significant association. Additional research is needed to confirm it. The role of the polymorphic variant *MYC* (rs4645948) in determining the clinical phenotype of MS remains to be found.

Conclusion. This study, conducted on a homogeneous sample of patients with RMS, showed that polymorphic variants of the *PVT1* locus affect the severity of MS, with a more pronounced association observed for SNP rs2114358. Undoubtedly, the data obtained need to be replicated in independent groups of patients. We hope that after the appropriate validation, they can be used for the compilation of panels of prognostic markers that allow to predict the rate of progression of MS in the early stages of the disease.

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Received/Reviewed/Accepted
15.05.2020/22.06.2020/1.07.2020

Conflict of Interest Statement

The investigation has been supported by the Russian Foundation for Basic Research under Research Project №19-315-51003. There are no conflicts of interest. The authors are solely responsible for submitting the final version of the manuscript for publication. All the authors have participated in developing the concept of the article and in writing the manuscript. The final version of the manuscript has been approved by all the authors.

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