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Original article

The effect of galanin gene polymorphism rs948854 on the severity of multiple sclerosis: A significant association with the age of onset

Victoria Lioudyno[a,*], Irina Abdurasulova[a], Alexander Tatarinov[a], Irina Nikiforova[b], Alexandr Ilves[b], Elena Ivashkova[b], Igor Stoliarov[b], Gennadij Bisaga[c], Victor Klimenko[a]

[a] Federal State Budgetary Scientific Institution "Institute of Experimental medicine", 197376, Akad. Pavlov str. 12, St. Petersburg, Russia
[b] N.P. Bechtereva Institute of the Human Brain of the Russian Academy of Sciences, 197376, Akad. Pavlov str. 9, St. Petersburg, Russia
[c] Almazov National Medical Research Center, 197341, Akkuratov str. 2, St. Petersburg, Russia

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ABSTRACT

Background: Data on genetic markers that determine the prognosis of multiple sclerosis (MS) is still limited. The association between galanin gene polymorphism rs948854 and prognosis of MS had been demonstrated earlier. *Objectives:* To confirm earlier findings in a distinct from the previously studied cohort of patients, and to further characterized the rs948854 polymorphism as one of the candidates for the risk stratification in patients with MS. *Methods:* To assess the rate of disease progression, the MS severity score (MSSS) and Age Related Multiple Sclerosis Severity (ARMSS) score were used, along with the Progression Index (PI). *Results:* The significant association of a minor allele of rs948854 polymorphism with the severity of the course of multiple sclerosis was revealed, confirming earlier findings. An increase in the proportion of patients with a MSSS > 5 (high rate of progression) was observed among the minor G allele carriers (genotypes AG and GG) compared to patients with AA genotype. Furthermore, the age at onset correlated with the MSSS value only in the group of minor allele carriers and the effect of a minor allele appeared only in patients with the late age at onset (> 30 years). *Conclusion:* Collectively, our data support the contribution of galanin gene polymorphism rs948854 to the mechanisms of adverse course of the disease in the late onset MS.

1. Introduction

Multiple sclerosis (MS) is a multifactorial neurodegenerative disease characterized by a progressive disability due to a chronic inflammatory reaction, demyelination and axonal degeneration (Compston and Coles, 2008). A search for biomarkers is a promising new approach that would help to predict the severity of the MS course and provide better understanding of the mechanisms of the MS progression.

A number of studies have confirmed the involvement of genetic factors in the etiology and pathogenesis of MS. At present, more than 200 loci, mostly in immune-related genes, are known to be associated with the risk of MS development (Parnell and Booth, 2017). It is widely accepted that a cumulative burden of risk genes forms a vulnerable background that can allow the disease to develop under the action of an environmental trigger (e.g., stress, infection), whereas the contribution of a single gene is not as large as in monogenic diseases. It is important to notice that recent GWAS studies failed to find any significant effect of the MS risk genes on the course of the disease, showing no overlap between the risk genes and the disease-modifying genes (George et al., 2016; Sawcer et al., 2011). Moreover, currently available data on the genetic factors influencing MS prognosis are obtained in individual populations with each study including a few polymorphisms, making it difficult to compare the results between these studies. Therefore, the search for genetic markers remains of high relevance for the risk stratification and personalized treatment development at the early stages of the disease.

The gene encoding the neuropeptide galanin is one of the non-immune genes that may affect progression of multiple sclerosis. Galanin is a neuropeptide whose protective effects are shown in various models of CNS damage, e.g., extensive activation of the glutamatergic system caused by the administration of glutamate NMDA receptor agonists (Elliott-Hunt et al., 2004; Webling et al., 2016), ischemic lesions (Hwang et al., 2004), and experimental models of demyelinating diseases (Wraith et al., 2009; Zhang et al., 2012). An explicit elevation of galanin production was revealed in the foci of demyelination both in the study of postmortem brain samples from MS patients and in the

GAL gene polymorphism rs948854 has a prognostic value in late-onset MS.

* **Corresponding author**.
E-mail address: vlioudyno@mail.ru (V. Lioudyno).

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experimental autoimmune encephalomyelitis (EAE) model of MS in rodents (Wraith et al., 2009). Importantly, over-expressing galanin-transgenic mice were found to be resistant to the EAE induction, whereas mice with loss-of-function mutations in the galanin or GalR2 genes have a severe course of EAE. These data support a role of galanin in alleviation of the disease progression.

Our previous findings demonstrated that the rs948854 polymorphism in the promoter region of the human galanin gene affects both the susceptibility to MS and the rate of disease progression, measured as a progression index (Lioudyno et al., 2017). Importantly, the polymorphism impact on vulnerability to MS was multidirectional for male and female patients, whereas the accelerating effect of a minor allele on MS progression and severity was unidirectional for patients of both sexes.

Based on these findings and given that rs948854 polymorphism is a common genetic variant with the MAF = 0.3187 (https://www.ncbi.nlm.nih.gov/snp/?term=rs948854) and the 1:1 ratio of alleles in MS cohort, we propose that it may be considered as one of the potential genetic markers for the risk stratification in patients with multiple sclerosis. Thus, to further characterize the role of rs948854 polymorphism in MS, we performed a study aimed to: (1) validate our previous findings using a different cohort of MS patients; (2) test whether the association of rs948854 polymorphism with the severity of the disease course can be confirmed using the MSSS algorithm that is accepted for studying the genetic factor's associations with disease progression; (3) establish the contributions of age and genetic sex to the rs948854 influence on the MS progression; (4) investigate the relationships between rs948854 genotype and the content of serum markers (tumor necrosis factor α (TNF-α) and phosphorylated neurofilament H (pNF-H)) of neurodegeneration and inflammatory process in patients with MS.

2. Patients and methods

2.1. MS patients

110 MS patients (66 women and 44 men) were enrolled in this study. The MS diagnosis was confirmed in accordance with the McDonald criteria (Polman et al., 2005). All patients were under ambulatory care in clinical departments of FSBSI “Institute of Experimental medicine” and N.P. Bechtereva Institute of the Human Brain. Current disability status was estimated by Kurtzke Expanded Disability Status Scale (EDSS). The patients were divided into five groups based on the information about their disease-modifying therapies (DMT) which was available from medical records. These groups included patients taking (1) Copaxone, (2) Fingolimod, (3) Laquinimod, (4) no DMT, and (5) different DMTs during the course of the disease. Patients with long-term history of steroid therapy were not included in this study.

The clinical characteristics of all MS patients are shown in Table 1. The study was approved by the local ethics committee, and written informed consent has been obtained from each patient. The duration of the disease less than one year was considered to be an exclusion criterion, since in this case it is difficult to determine the rate of the disease progression.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

2.2. Assessment of neurological status of patients and MS progression

The Multiple Sclerosis Severity Score (MSSS) was used to measure the rate of disability accumulation (Roxburgh et al., 2005). The original MSSS was determined based on EDSS score outside of the relapse period, age and disease duration in: https://aliman.shinyapps.io/ARMSS/. MSSS was assessed as a continuous variable and as 2 dichotomous variables: MSSS of ≤ 5 vs. > 5 (slower progression vs. fast progression) and MSSS of < 2.5 vs. ≥ 7.5 (extreme ends of the MSSS distribution, indicating benign and severe variants of MS) (George et al., 2016). In addition to MSSS, all subjects were scaled according to ARMSS (Age Related Multiple Sclerosis Severity) score, which considers chronological age and not dependent on MS duration and age of onset.

Table 1
The demographic and clinical characteristics of enrolled MS patients.

	Mean ± SE	Min	Max
Age (years)	40.9 ± 1.03	20	61
Age of onset (years)	30.1 ± 0.95	11	56
Duration of MS (years)	10.8 ± 0.77	1	30
DMT (C,F,L,N,D)	22/23/24/21/20	–	–
EDSS	3.5 ± 0.15	1	7.5
PI	0.66 ± 0.08	0.09	3.0
MSSS	4.67 ± 0.23	0.6	9.6
ARMSS	5.72 ± 0.19	0.8	9.7

Abbreviations: DMT – disease modifying therapy; C – copaxone; F – fingolimodum; L – Laquinimod; N – no therapy; D – different DMTs during the course of the disease; EDSS – Expanded Disability Status Scale; PI – Progression Index; MSSS – Multiple Sclerosis Severity Score; ARMSS - Age Related Multiple Sclerosis Severity Score.

As an alternative method for estimating the rate of disease progression, a progression index (PI) was calculated as ratio of an individual's EDSS to disease duration (Poser et al., 1982). This allowed us to directly compare the results with the findings of our previous study that used the same method (Lioudyno et al., 2017).

To analyze whether the rs948854 genotype affects the disease progression the results from both homozygous and heterozygous minor G-allele carriers were combined and compared with the data obtained from homozygous AA-carriers.

2.3. DNA extraction and genotyping

Peripheral blood samples (5 ml) were collected in tubes with EDTA. DNA extraction was performed using a DNA isolation kit (DNA-sorb B; Next-Bio, St. Petersburg, Russian Federation). For the genotyping of rs948854 polymorphism, a previously described procedure was applied (Lioudyno et al., 2017). The primers were designed based on the GenBank genomic sequence (accession number NT_167190.1). The rs948854 polymorphism is located in the promoter region of the GAL gene and is a single nucleotide substitution (A/G) with minor allele frequency of 0.32 (https://www.ncbi.nlm.nih.gov/snp/rs948854).

2.4. Estimation of the serum markers of neuroinflammation and axon degeneration

The serum levels of tumor necrosis factor α (TNF-α) and phosphorylated neurofilament H (pNF-H), which both are associated with the activity of the pathological process in MS (Ljubisavljevic et al., 2016; Ji et al., 2016; Probert, 2015), were measured by enzyme-linked immunosorbent assay (ELISA). Blood samples were taken at the time of clinical remission.

2.5. Statistical analysis

In case when MSSS was analyzed as a continuous variable, one-way ANOVA (for genotype, group or therapy effect) or two-way ANOVA (for genotype/sex and genotype/therapy effects) was used for statistical analysis. For multiple pairwise comparisons between groups of patients, LSD *post-hoc* analysis was applied. In order to compare the proportions when a dichotomously divided MSSS scores needed to be processed, two-tail χ^2-test (with the Yates correction for groups with $n < 10$) and Fisher's exact probability test (for the groups with $n < 5$) were used. $p < 0.05$ was considered statistically significant.

One-way ANOVA and factorial ANOVA were used to analyze whether the DMT's type affects the association of the genotype with the disease progression. For this analysis, the patients were divided into five groups depending on whether they were treated with (1) Fingolimod, (2) Copaxone, (3) Laquinimod, (4) no DMT or (5) different DMTs during the course of the disease. To assess the influence of several factors on MS progression (genotype, age at onset, genetic sex) multiple regression analysis was used. Pearson's correlation coefficient was counted to assess the association between the MS severity (measured as MSSS) and onset age. The nonparametric gamma coefficient was calculated to analyze the correlation between the TNF level and MSSS.

The nonparametric Mann-Whitney *U* test was used for statistical analysis of the data with a distribution other than normal (the clinical data: age, age of MS onset, disease duration, EDSS score and data about the serum level of TNF-α and pNF-H).

All statistical analyses were performed in Statistica 10.0.

3. Results

3.1. The association between the rs948854 polymorphism and disease progression

The clinical characteristics of MS patients are shown in Table 1. All patients were genotyped for rs948854 polymorphism. MSSS value, as well as ARMSS and PI for each patient were calculated. The values of MSSS were analyzed in each subgroup as a continuous variable and as 2 dichotomous variables. All parameters were compared between groups divided according to either genotype only or both, genotype and genetic sex. When the absolute values of MSSS, ARMSS and PI were compared between groups, significant difference in mean PI, but not in MSSS and ARMSS were found between the groups by genotype (Table 2), confirming our previous findings (Lioudyno et al., 2017). However, as Table 3 shows, the analysis revealed the significant difference between genotypes in the distribution of patients with MSSS ≤ 5 (slowly progressing) and > 5 (rapidly progressing). The prevalence of patients with a high rate of progression (55.6%) was observed in the subgroup of carriers of the minor G-allele and only 28.6% of these patients were among those with AA genotype. Consistently, the presence of a minor G-allele increased the risk of rapid MS progression almost twice ($p = 0.004$; Risk ratio = 1.94).

After the stratification by genetic sex, the difference between genotypes in MSSS values distribution (≤5 vs > 5) was significant in female patients ($p = 0.025$; Risk ratio = 2.26), whereas it lost significance in male patients ($p = 0.358$; Risk ratio = 1.48). The trend towards an increase in a subset of patients with a rapid disability accumulation among G-allele carriers, compared to those with AA genotype (57.7% vs 38.9% with the Risk ratio = 1.48) was, however, present in male patients group. Interestingly, no interaction between the genotype and genetic sex was found when its influence on MSSS range (≤5 vs > 5) was analyzed (ANOVA, $F_{genotype} = 5.059$; $p = 0.027$; $F_{genotype/sex} = 0.225$; $p = 0.64$). In contrast, significant genotype-sex interaction was found when MS progression was assessed using ARMSS ($F_{genotype/sex} = 5.287$; $p = 0.024$).

The analysis of distribution of extreme MSSS values (< 2.5 and ≥7.5) did not reveal any differences between rs948854 genotypes as in a whole group or in subgroups after the stratification by genetic sex.

3.2. The influence of DMT type, age of onset, genetic sex, and rs948854 genotype on MS progression

No differences in disease progression (by MSSS measures) were revealed between groups of patients divided according to their DMT type. There was also no interaction between the genotype and DMT type ($F_{therapy} = 1.807$; $p = 0.118$; $F_{genotype/therapy} = 1.541$; $p = 0.184$). These results were consistent with the results of analysis that utilized PI measurements.

Multiple regression analysis was performed to better understand the combined effect of genotype and such characteristics as an age at onset and a sex of patient on MS progression. The analysis showed significant influence of age at onset and genotype to MSSS range–≤5 vs > 5 ($R^2 = 0.128$; $p = 0.002$; $p_{genotype} = 0.008$ (CL: 0.066–0.426); $p_{age\ of\ onset} = 0.022$ (CL: 0.030–0.386), whereas no effects of the genetic sex were revealed.

Interestingly, a positive correlation was found between the age of onset and MSSS value when the analysis was conducted for the whole group ($r = 0.26$; $p = 0.006$, $N = 110$; Fig. 1A). After dividing the group by genotype, the correlation was found to be even stronger for the minor allele carriers ($r = 0.39$; $p = 0.004$, $N = 56$; Fig. 1B) but was not observed in patients with AA genotype ($r = 0.14$; $p = 0.282$, $N = 54$; Fig. 1C).

Based on the results of multiple regression and correlation analysis, we categorized the MS patients into four groups: according to the rs948854 genotype and the age at onset (up to 30 years old and over 30 years old). The comparison of MSSS in these groups using the one-way ANOVA revealed significant differences ($F_{group} = 3.83$; $p = 0.012$; $P = 0.81$; Fig. 2). The highest mean MSSS value (5.9 ± 0.45) was found in the group of minor G-allele carriers with the late age of onset. MSSS in this group was significantly higher than the MSSS in minor G-allele carriers with the earlier age of onset (LSD *post-hoc:* $p = 0.01$; Fig. 2). Importantly, the MSSS values were not different significantly between the patients with early and late age of MS onset in the group of AA genotype carriers. Finally, there were no differences in MSSS between genotypes in groups of MS patients with early age of onset.

3.3. The analysis of serum TNF-α and pNF-H in MS patients

To investigate whether the effect of galanin polymorphism on MS severity is linked to pro- inflammatory process and/or neurodegenerative mechanisms of MS, the levels of TNF-α and pNF-H, which may reflect disease activity, were measured in the patient's serum. First, a

Table 2
The summary statistics for MS patients grouped by genetic sex and rs948854 polymorphism genotype.

	MS patients ($N = 110$)		Male MS patients ($N = 44$)		Female MS patients ($N = 66$)	
	AA genotype ($N = 56$)	AG,GG genotypes ($N = 54$)	AA genotype ($N = 18$)	AG,GG genotypes ($N = 26$)	AA genotype ($N = 38$)	AG,GG genotypes ($N = 28$)
Age (years)†	41.6 ± 1.46	40.1 ± 1.46	37.0 ± 2.34	39.1 ± 2.06	43.8 ± 1.75	41.0 ± 2.08
Age of onset (years)†	29.6 ± 1.43	30.6 ± 1.25	25.9 ± 2.20	30.9 ± 1.85	31.4 ± 1.77	30.4 ± 1.72
Duration of MS (years)†	11.9 ± 1.04	9.5 ± 1.13	11.1 ± 1.62	8.3 ± 1.55	12.4 ± 1.33	10.6 ± 1.64
EDSS†	3.46 ± 0.20	3.45 ± 0.23	3.78 ± 0.40	3.25 ± 0.34	3.3 ± 0.22	3.64 ± 0.31
PI†	0.46 ± 0.08	0.88 ± 0.13*	0.47 ± 0.09	0.99 ± 0.22	0.35 ± 0.04	0.77 ± 0.15
MSSS†	4.30 ± 0.29	5.05 ± 0.34	4.86 ± 0.56	5.1 ± 0.49	4.04 ± 0.34	5.0 ± 0.49
ARMSS†	5.60 ± 0.28	5.85 ± 0.27	6.60 ± 0.52	5.66 ± 0.40	5.12 ± 0.31	6.03 ± 0.36

† Data presented as mean ± SD.
* Significant difference between genotypes (AA vs AG,GG); $p < 0.05$.

Table 3
The comparative analysis of distribution of frequencies in the median (≤5 vs > 5) and in the extreme (< 2.5 vs ≥7.5) ends of MSSS without the separation of patients by the MS age of onset.

		MS patients (N = 110)		Male MS patients (N = 44)		Female MS patients (N = 66)	
		AA genotype (N = 56)	AG,GG genotypes (N = 54)	AA genotype (N = 18)	AG,GG genotypes (N = 26)	AA genotype (N = 38)	AG,GG genotypes (N = 28)
MSSS	≤ 5,% (n)	71.4 (40)	44.4 (24)	61.1 (11)	42.3 (11)	76.3 (29)	46.4 (13)
	> 5,% (n)	28.6 (16)	55.6 (30)	38.9 (7)	57.7 (15)	23.7 (9)	53.6 (15)
Statistical data		$\chi2$ = 8.227; p = 0.004		$\chi2$ = 0.846; p = 0.358		$\chi2$ = 4.998; p = 0.025	
		Risk ratio = 1.94		Risk ratio = 1.48		Risk ratio = 2.262	
		CL [1.206–3.136]		CL [0.762–2.888]		CL [1.161–4.406]	
MSSS	< 2.5, % (n)	21.4 (12)	24.1 (13)	11.1(2)	23.1(6)	26.3(10)	28.6(8)
	≥7.5, % (n)	10.7(6)	16.7(9)	11.1(2)	15.4(4)	10.5(4)	17.9(5)
Statistical data		$\chi2$ = 0.027; p = 0.869		p > 0.99†		p = 0.089†	
		Risk ratio = 1.23		Risk ratio = 0.80		Risk ratio = 1.35	
		CL [0.538–2.798]		CL [0.232–2.763]		CL [0.459–3.950]	

† According to Fisher exact test.

positive correlation was found between the serum TNF-α level and the MSSS range (G = 0.45; p = 0.019), confirming the role of TNF-α in the pathogenesis of multiple sclerosis. After entering the rs948854 genotype and gender in a correlation analysis, the serum TNF-α levels still showed a correlation with MSSS score at the same level of significance (G = 0.45; p = 0.019). We also found no difference when comparing the median TNF-α levels in patients with different rs948854 genotypes (U = 86.5; p = 0.451) (Table 4). However, the values exceeding the normal level for TNF-α (19 pg/ml) were observed in the carriers of minor G-allele more often than in patients with the AA genotype (in 63.6% and 26.3%, accordingly). For pNF-H, no significant correlations were revealed, neither with MSSS score, nor with a rs948854 genotype (Table 4).

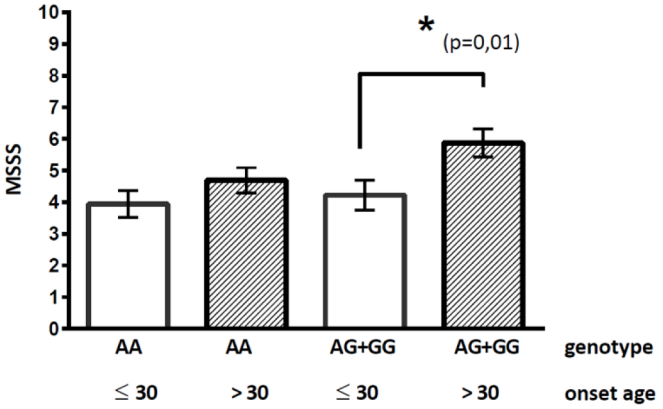

Fig. 2. The rate of MS progression in groups of patients separated by rs948854 polymorphism genotype (AA vs AG + GG) and onset age (≤ 30 vs > 30 years). Data are shown as mean ± SD ANOVA: Fgroup = 3.78; p = 0.013; P = 0.80 *-significant difference between groups (LSD post-hoc: p = 0.01).

4. Discussion

The present study confirmed and extended our earlier findings about the contribution of galanin polymorphism rs948854 to the progression of multiple sclerosis. As we demonstrated before (Lioudyno et al., 2017), rs948854 polymorphism affected both, susceptibility to MS and the rate of disease progression measured as a progression index (PI). In the current study the analysis of MS progression was performed on different cohort with larger sample of patients. Using the same methodology, we replicated our earlier finding as well as extended it utilizing MSSS algorithm that is considerably more powerful and suites specifically for studying the associations between genetic factors and the MS progression. A number of publications validated this robust method for analyzing the MS severity

Table 4
The ELISA-measured levels of TNF-α and pNF-H in the serum of MS patients.

	AA genotype (N = 21)	AG,GG genotypes (N = 13)
TNF-α (pg/ml)†	11.0 [0; 20.0]	20.0 [0; 28.5]
pNF-H (ng/ml)†	6.25 [2.43; 8.13]	2.90 [1.80; 8.30]

† Data presented as median with interquartile range [1Q; 3Q].

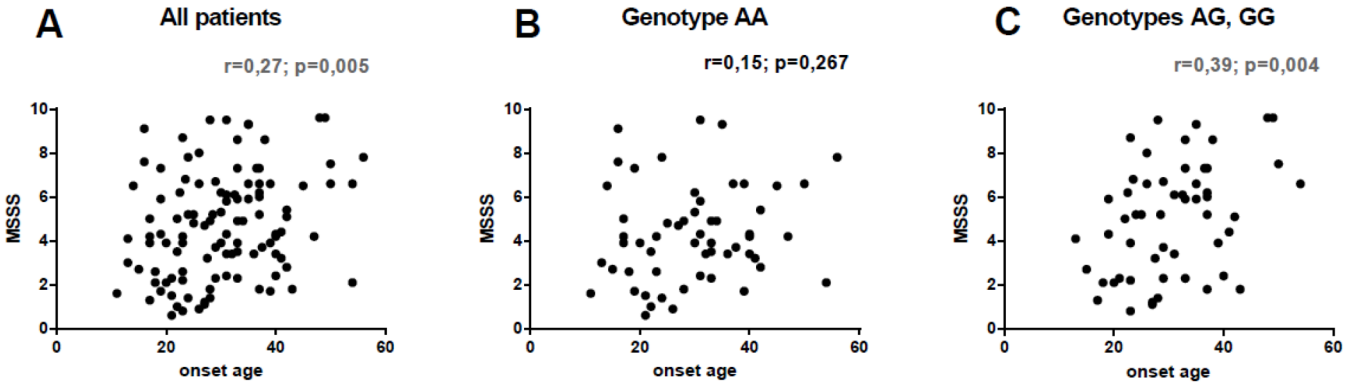

Fig. 1. The association between the onset age and disease progression.

(Roxburgh et al., 2005; Pachner, Steiner I., 2009; Daumer et al., 2009). Applying the MSSS, our analyses revealed an almost two-fold increase in the risk of rapid MS progression for the minor allele carriers. The significant differences between the genotypes in the distribution of patients with slowly progressing (MSSS $\leq$ 5) and rapidly progressing (MSSS > 5) course were found. However, no differences between the genotypes were found when the MSSS extremes (< 2.5 vs $\geq$7.5) were analyzed. We attribute these to the small number of patients with a benign and severe course of the disease (extreme ends of MSSS distribution).

The ARMSS, an alternative scale for assessing the MS progression, which is not dependent on disease duration and the age at onset, was also used (Manouchehrinia et al., 2017).

Furthermore, we investigated whether the age and/or the biological sex can modify the effects of galanin rs948854 polymorphism on MS severity. Our analysis revealed that the rs948854 polymorphism significantly affects the course of the disease in the subgroup of patients with the late age of onset. There is a general agreement that, prognosis of the MS depends on the onset age, with the least favorable prognosis for the patients who manifested disease after the age of 30–35 years old (Confavreux and Vukusic, 2006; Scalfari et al., 2011; Ramachandran et al., 2014; Tedeholm et al., 2015). Accordingly, multiple regression analyses performed in our study revealed significant effect of the onset age on the MSSS value, in addition to a finding of positive correlation between the MSSS value and the age of onset. This effect was carried by a subgroup of patients with minor allele for rs948854, as the subsequent subgroup analysis established association between the MSSS value and the age of onset in minor allele carriers but not in the patients with AA genotype. The additional subgroup analysis by the age of onset (< 30 vs. > 30 years) revealed the highest mean MSSS value in the group of minor allele carriers with the late age of onset.

The further studies are needed to answer the question of why the presence of a minor allele of rs948854 polymorphism becomes significant for MS patients past a certain age. Several mechanisms that may underlie poor prognosis in late onset MS patients had been proposed. They include an imbalance of the immune system, a shift towards pro-inflammatory reactions, as well as a decrease in neuroplasticity and reparative ability (Musella et al., 2018). In MS, galanin affects the oligodendrocyte's survival, exerts trophic and proliferative effects and stimulates the myelin formation (Gresle et al., 2015; Lyubetska et al., 2015). In addition, overexpression of galanin in microglia is considered protective, as it prevents the aggravation of pro-inflammatory activity (Wraith et al., 2009). Collectively, these data suggests that galanin controls pathophysiological process reducing neuroinflammation and protecting from demyelination. Given that rs948854 is located in the promoter region of the GAL gene and likely affects its transcription and expression, the lack of expression due to A to G substitution may aggravate the age-related inability to cope with pathological process triggered by neuroinflammation. Although there is no direct evidence for the functional regulation of GAL promoter strength by A to G substitution in rs948854, our findings that demonstrate its effect on MS severity in patients with later age of onset support the later hypothesis. Several other studies demonstrated phenotypic effect of the G-allele in rs948854 that is consistent with our results. It had been shown that rs948854 is associated with the activity of HPA-axis and has an impact on severity in female patients with the anxiety disorder (Unschuld et al., 2010). It also plays a role as susceptibility factor in opioid addiction (Beer at al., 2013) and depressive disorders (daMachado et al., 2018).

Our analyses of association between the rs948854 polymorphism and MS progression revealed higher prevalence of patients with the fast rate of progression among G-allele carriers. The fact that, after stratification for sex, the differences in MSSS values distribution between genotypes were significant in female patients, but not in males, suggests that biological sex may modify mechanism(s) underlying the association between rs948854 genotype and MS progression. The latter is consistent with our earlier findings (Lioudyno et al., 2017) that utilized PI as a measure for MS progression, as well as with the observed trend towards an increase in a subset of male patients with a rapid MS progression among G-allele carriers compared to those with AA genotype. Furthermore, the link between the rs948854 genotype and the severity of the MS was also confirmed by using ARMSS. With the latter approach, a significant interaction had been found between carrying a minor allele and the genetic sex of the patients.

Importantly, the results of this study are obtained using a population-based cohort of patients. Therefore, future studies that will extend the analysis to different geographical populations are required. Given the well-established role of galanin in the regulation of neuronal viability and axonal integrity, as well as its link to MS, it is compelling to hypothesize that similar results will be obtained using cohort(s) from different populations.

In the present work, we also aimed to elucidate the mechanism(s) underlying the effect of rs948854 polymorphism on MS progression. To test whether rs948854 is linked to neuroinflammation, we analyzed the serum levels of pro-inflammatory cytokine TNF-α which is known to play an important role in the pathogenesis of multiple sclerosis (Probert, 2015; Ji et al., 2016; Rizzo et al., 2018). We further analyzed the serum levels of phosphorylated neurofilament H (pNF-H) as a marker of axonal loss (Ljubisavljevic et al., 2016). Our results showed that neither the TNF-α levels, nor the levels of pNF-H were different between genotypes. However, the analysis of TNF-α in serum revealed a trend: it was elevated in more than a half of the carriers of the minor allele. Although, it cannot be concluded, due to a small sample size, whether rs948854 is associated with serum level of TNF-α, its elevation in a subgroup of patients with minor allele may indicate the involvement of galanin into the regulation of TNF-mediated neuroinflammation in MS. Since an increase in the serum TNF-α level may be associated with other factors, including the polymorphism of TNF-α gene itself, further studies are needed to directly adress the role of galanin and galanin gene polymorphisms in the complex mechanisms of neuroinflammation in MS.

In conclusion, our study, applying several independent approaches (PI, MSSS, ARMSS), demonstrated association of rs948854 polymorphism with the severity of the MS and, analyzing different cohort of MS patients, replicated and extended our earlier findings (Lioudyno et al., 2017). In addition, the current study demonstrated that minor rs948854 allele carriers with later onset of MS, have the highest risk of adverse course of the disease.

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CRediT authorship contribution statement

Victoria Lioudyno: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Irina Abdurasulova:** Formal analysis, Investigation. **Alexander Tatarinov:** Data curation. **Irina Nikiforova:** Data curation. **Alexandr Ilves:** Data curation, Formal analysis. **Elena Ivashkova:** Data curation, Formal analysis. **Igor Stoliarov:** Validation, Supervision, Writing - review & editing. **Gennadij Bisaga:** Validation, Writing - review & editing. **Victor Klimenko:** Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest regarding the publication of this article.

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