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ASSOCIATION OF SINGLE NUCLEOTIDE VARIANTS IN THE *DRD3* AND *LINGO1* GENES WITH THE DEVELOPMENT OF DRUG DYSKINESIAS IN PARKINSON'S DISEASE: RESULTS OF A PILOT STUDY

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Levodopa is the "gold" standard of pharmacotherapy for Parkinson's disease (PD). Levodopa has its own advantages, such as high efficiency at all stages of the disease, low incidence of side effects, availability, at the same time, long-term levodopa therapy is associated with the development of levodopa-induced dyskinesias (LID). Approximately one third of patients in the fifth year of illness already have LID; by the 10th year of illness, almost all patients have this disorder. LID can be associated with gene polymorphisms, the products of which are involved in the metabolism of levodopa. The aim was to study the association of single nucleotide variants (SNV) rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) with the development LID in PD. The study included 47 patients with PD, 21 (44.7%) men and 26 (55.3%) women. The average age was 69.0 ± 7.67 years. Patients with a mixed form of PD predominated (72.3%). The average duration of the disease was 5.94 ± 4.09 years. Results. Patients with PD in both groups (with and without LID) did not differ in age, gender and ethnicity, stage of disease, non-motor symptoms, and degree of cognitive impairment. At the same time, patients with LID showed frequent development of motor fluctuations, a longer duration of levodopa therapy, and a higher levodopa equivalent daily dose. Analysis of the effect of DRD3 (Ser9Gly, or rs6280) and *LINGO1 (rs9652490)* polymorphisms on the development of LID in PD was not found. Conclusion. The results of a pilot study indicate the absence of a predictive role of the carriage of SNV rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) on the development of LID in PD patients living in the Republic of Sakha (Yakutia). However, the authors do not exclude the influence of a small sample size on the results of the associative genetic study.

Keywords: Parkinson's disease, movement disorder, levodopa, levodopa-induced dyskinesia, side effect, pharmacogenetics, personalized medicine, single nucleotide variants, *DRD3, LINGO1.*

Introduction. Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the world [1, 24]. Levodopa remains one of the effective drugs in PD pharmacotherapy [5, 19, 24]. Levodopa has advantages such as high efficacy at all stages of the disease, low incidence of side effects, availability, however long-term therapy is associated with the development of levodopa-induced dyskinesias (LID) – choreiform hyperkinesias that result from overstimulation of dopamine receptors [4, 14]. The development of LID is mainly based on the continuing death of nigrostiary neurons with a loss of their "buffer capacity" [3]. Approximately one third of patients already have LID by the fifth year of the disease, and by the 10th year of the disease all patients may have this complication [16].

It has been established that LID can be associated with gene polymorphisms, the products of which are involved in the levodopa metabolism. Thus, A allele of COMT gene (rs4680), AA genotype of MAO-B gene (rs1799836, A644G), T allele SLC6A3 gene (653 + 4065C>A, rs393795), allele 15 of DRD2 gene (CAnSTR) and other polymorphisms were associated with earlier development of LID in patients with PD [7, 9, 12, 22, 27]. At the same time, there is a limited number of studies on the effect of mutations in the DRD3 and LINGO1 genes on the development of LID.

The DRD3 gene encodes the D3 subtype of dopamine receptors, which are in the presynaptic membranes of nerve cells (autoreceptor) and in postsynaptic membranes. A large number of D3 receptors are localized in the limbic system, which is associated with cognitive, emotional and endocrine functions. Carriage of single nucleotide variants of the DRD3 gene can influence the formation of a therapeutic response to antiparkinsonian and antipsychotic drugs, the development of side effect and contribute to the development of alcohol, nicotine and heroin addiction [11, 30].

The aim was to study the association of single nucleotide variants (SNV) rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) with the development of LID in PD.

Material and methods. The object of the study was patients with PD, observed at the Center for Extrapyramidal Disorders and Botulinum Therapy of the Clinic of the M.K. Ammosov North-Eastern Federal University (Yakutsk). The research protocol was approved by the Local committee on biomedical ethics of the Yakutsk Scientific Center for Complex Medical Problems (Protocol No. 43, November 9, 2016).

The study included 47 patients with PD who underwent careful history and clinical selection using inclusion and exclusion criteria. Inclusion criteria: 1) clinically reliable diagnosis of PD according to the MDS criteria [15]; 2) the ability to complete the full scope of research; 3) the patient's consent to genetic analysis. Exclusion criteria: 1) cases of secondary and tertiary parkinsonism; 2) failure to

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Table 1

Nucleotide sequence of primers

Single nucleotide variants, gene, localization	Nucleotide sequence of primers
rs6280, DRD3, 3q13.31	Forward: GTAGGAGAGGGCATAGTAG Reverse: CTGTCTCCTCACAGGAAG
rs9652490, LINGO1, 15q24.3	Forward: AGGAGAAGAAAAGAGGTG Reverse: GGAGAATAGGAAGGAGAC

Table 2

Clinical and anamnestic comparison of patients with Parkinson's disease and with / without levodopa-induced dyskinesia

Parameter	PD – LID	PD + LID	p-level
Age, year	69.0 [64.0; 75.8]	68.0 [62.0; 75.0]	0.473
Men / women, abs	20 / 20	1 / 6	0.112
Yakut / Russian, abs.	25 / 15	4 / 3	0.55
Duration of illness, year	4.5 [2.0; 7.75]	6.0 [5.0; 12.0]	0.065
The stage of the disease on the Hoehn-Yahr scale	3.0 [2.0; 3.0]	3.0 [3.0; 3.0]	0.567
Number of NMSs by NMSQuest	10.5 [5.25; 13.75]	7.0 [4.0; 9.0]	0.119
MoCA, score	22.5 [16.0; 25.75]	24.0 [17.0; 27.0]	0.6
MMSE, score	27.5 [23.25; 30.0]	29.0 [24.0; 30.0]	0.465
FAB, score	17.0 [11.25; 18.0]	17.0 [13.0; 18.0]	0.654
3 part UPDRS, score	46.5 [29.25; 56.0]	27.0 [24.0; 53.0]	0.22
Motor fluctuation, %	17.5	57.1	0.04
Levodopa-therapy, %	82.5	100	0.57
Levodopa therapy experience, years	2.0 [1.0; 3.0]	4.0 [3.0; 6.0]	0.007
LEDD, mg per day	675.0 [500.0; 787.5]	1150.0 [862.0; 1187.0]	< 0.001

Abbreviations: PD¬ – LID ¬– PD patients without LID; PD + LID – PD patients with LID; NMS - non-motor symptoms; NMSQuest – non-motor symptoms questionnaire scale; MoCA – Montreal Cognitive Assessment; MMSE – Mini-Mental State Examination; FAB – Frontal Assessment Battery; UPDRS – Unified Parkinson's Disease Rating Scale; LEDD ¬– levodopa equivalent daily dose.

Table 3

Relationship of *DRD3* and *LINGO1* gene polymorphisms with levodopa-induced dyskinesias in Parkinson's disease, abs. (%)

Genotype	PD – LID	PD + LID	χ^2	p	OR (95 % CI)
Ser9Gly polymorphism *DRD3* gene					
CC *CT* *TT*	3 (7.5) 8 (20) 29 (72.5)	1 (14.3) 0 6 (85.7)	1.86	0.394	2.06 (0.18–23.2) Н/Д 2.28 (0.24–21.1)
rs9652490 polymorphism *LINGO1* gene					
CC *CT* *TT*	9 (22.5) 21 (52.5) 10 (25)	1 (14.3) 4 (57.1) 2 (28.6)	0.243	0.886	0.57 (0.06–5.41) 1.2 (0.24–6.1) 1.2 (0.2–7.18)

complete the full scope of the study; 3) refusal of the patient or legal representative from genetic research; 4) non-use of antiparkinsonian drugs.

There were 21 men (44.7%) and 26 women (55.3%). The average age of patients was 69.0 ± 7.67 years, the median age was 69.0 [64.0; 75.0] years. Most of the patients were of the Yakut ethnic group (29 people, 61.7%), 18 people (38.3%) were of the Russian ethnic group. Patients with a mixed form of PD predominated (72.3%). The average duration of the disease was 5.94 ± 4.09 years, the median duration of the disease was 5.0 [3.0; 9.0] years. The average stage of the disease according to the Hoehn-Yahr scale was 2.78 ± 0.87, the median was 3.0 [2.0; 3.0].

DNA-sorb-V reagent kit (Diagnost, Russia) was used for DNA isolation. DNA genotyping was performed on a CFX96 Real-Time PCR amplifier (Bio-Rad Laboratories, USA) using an amplification reagent kit (Testgen, Russia). The amplification program included the first denaturation at 95 ° C for 2 minutes, then 40 cycles at 94 ° C for 10 seconds and at 60 ° C, 62 ° C and 58 ° C for 20 seconds, the fluorescence signal was measured at the second stage. Table 1 shows the nucleotide sequence of the forward and reverse primers of the studied polymorphisms.

Statistical analysis was performed using SPSS Statistics 25. Descriptive statistics are given as median and 25th and 75th quantiles (Me [Q25; Q75]). Data analysis for two independent groups was carried out by the Mann – Whitney U-test. For the analysis of nominal data, we used four-field contingency tables using Pearson's χ2 test and Fisher's test. The ratio of the frequencies of genotypes and allelic variants of genes was checked for compliance with the Hardy-Weinberg equilibrium. The frequencies of genotypes and alleles of each polymorphism were calculated as a percentage of their total number with the calculation of rela-

tive odds (OR) and 95% confidence interval (CI). The critical level of statistical significance for the two groups was determined at $p \leq 0.05$.

Results. LID were detected in 7 patients with PD (14.9%). Comparative analysis of clinical and anamnestic data of patients with and without PID is presented in Table 2.

According to Table 2, patients with PD in both groups (with and without LID) did not differ in age, gender, ethnicity, stage of disease, non-motor symptoms, and cognitive impairment. At the same time, patients with LID are more likely to have motor fluctuations, a longer duration of levodopa therapy, and a higher levodopa equivalent daily dose.

We studied the relationship of DRD3 and LINGO1 gene polymorphisms with LID in PD (Table 3) and did not find such an association.

Discussion. The dopamine D3 receptor gene (DRD3) is located on chromosome 3 (3q13.3) [10]. The rs620 (Ser-8Gly) polymorphism of the DRD3 gene is a replacement of C allele encoding serine with T allele encoding glycine. As a result, the affinity of the D3 receptor for dopamine decreases, and patients with Gly / Gly genotype require a higher dose of dopamine receptor agonists [9]. J.Y. Lee et al. found the effect of TT genotype of this polymorphism on the development of biphasic LID (OR 3.1; 95% CI 1.4–6.5) [13]. Similar results were obtained in patients with PD from Italy (OR 4.9; 95% CI 2, 0-12.2) [25].

The LINGO1 gene is located on chromosome 15q24 and encodes a transmembrane glycoprotein of the central nervous system that plays a role in the structural plasticity and survival of dopaminergic neurons [18, 29]. It has now been established that mutations in the LINGO1 gene are associated with the development of essential tremor [2, 20, 31]. About Parkinson's disease, the results are different. In a study by Carles Vilarino-Guell and colleagues involving 426 patients with PD, an increased risk of disease was reported with the carriage of AA genotype of the rs9652490 polymorphism [20]. On the contrary, a group of scientists from China, as a result of genotyping 425 patients with PD, did not reveal the effect of mutations in this gene on the development of the disease [26]. A similar result was obtained by Yih-Ru Wu and colleagues, who studied the effect of this polymorphism on 649 patients with PD from Taiwan and Singapore [17]. In 2020, Ting Gao and colleagues also reported that there was no effect of the rs9652490 polymorphism on the development of PD [8]. At the same time, we found no studies on the effect of mutations in the LINGO1 gene on the development of LD.

We did not find the effect of both the Ser9Gly (rs6280) polymorphism of the DRD3 gene and the rs9652490 polymorphism of the LINGO1 gene on the development of LID in PD patients.

One of the significant limitations of our study is a small sample, which allows us to classify this study as a pilot study. Despite this, it was rightly revealed that long-term levodopa therapy and a high daily dose of levodopa are statistically significantly associated with the development of LID. Therefore, the absence of a relationship between the development of LD in PD with the Ser9Gly (rs6280) polymorphisms of the DRD3 gene and rs9652490 of the LINGO1 gene should be taken as a true negative result.

Conclusion. The results of a pilot study indicate the absence of a predictive role of the carriage of SNV rs6280 (DRD3 gene) and rs9652490 (LINGO1 gene) on the development of LID in PD patients living in the Republic of Sakha (Yakutia). However, the authors do not exclude the influence of a small sample size on the results of the associative genetic study.

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