Original Article

Dissimilar mechanistic background of peripheral and orofacial hyperkinesia in patients with Parkinson’s disease and levodopa-induced dyskinesia

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Abstract

Introduction: Long-term levodopa treatment of Parkinson’s disease (PD) is frequently complicated by spontaneously occurring involuntary muscle movements called dyskinesia. The exact pathological mechanism of this complication has not yet been elucidated. We have previously demonstrated that in PD patients the vulnerability to develop peripheral but not orofacial dyskinesia is associated with the presence of two variants of the GRIN2A gene. Moreover, we have shown that in tardive dyskinesia (TD) orofacial dyskinesia is associated with other polymorphisms as compared with peripheral dyskinesia. In the present study we investigate whether the peripheral versus orofacial nature of levodopa-induced dyskinesia (LID) in PD can be explained by considering polymorphisms for dopaminergic and serotonergic receptors.

Materials and Methods: 101 Russian patients with PD (38M/63F) were examined. Genotyping was carried out on 19 SNPs for 3 neurotransmitter genes: 10 SNPs for DRD3 gene (rs11721264, rs167770, rs3773678, rs963468, rs7633291, rs2134655, rs9817063, rs324035, rs1800828, rs167771), 1 SNP for DRD4 gene (rs3758653), and 8 SNPs for HTR2C gene (rs6318, rs5946189, rs569959, rs17326429, rs4911871, rs3813929, rs1801412, rs12858300).

Results: Genotyping patients with PD and LID revealed that only rs3773678 (DRD3, dominant, p = 0.042) was associated with orofacial dyskinesia.

Conclusion: The findings of the current study are not related to LID in PD itself, but to other forms of orofacial dyskinesia in this patient group.

Abbreviations: PD – Parkinson’s disease; TD – Tardive dyskinesia; LID – Levodopa-induced dyskinesia; MSN – Medium Spiny Neuron; NMDA – N-methyl-D-aspartate; HD – Huntington’s disease; SNP – Single nucleotide polymorphism

Keywords:
Levodopa-induced dyskinesia; Parkinson’s disease; Dopaminergic receptors; Serotonergic receptors; Genetic variants

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Introduction

More than 45 years after its introduction levodopa still remains one of the most effective treatments for Parkinson’s disease (PD). However, long-term levodopa treatment of PD is frequently complicated by motor fluctuations and dyskinesia (Thanvi and Lo, 2004; Thanvi et al., 2007; Del Sorbo and Albanese, 2008). Several theories have been developed to explain the pathophysiology of this important treatment complication with the ultimate goal to develop treatments to prevent this side effect (Huot et al., 2013; Bargiotas and Konitsiotis, 2013; Cerasa et al., 2014). However, the exact pathological mechanism has not yet been elucidated.

Recently, we reported our serendipitous finding in a small group of patients we used as a control group in our study on tardive dyskinesia (TD) in patients with schizophrenia, that two variants of GRIN2A gene were strongly associated with levodopa-induced dyskinesia (LID) and not with TD (Ivanova et al., 2012; Loonen and Ivanova, 2013). It is important to note that these variants were already known to be associated with the age of onset of symptoms in patients suffering from Huntington’s disease (Arning et al., 2007). The GRIN2A gene encodes for a protein which is part of the ionotropic glutamatergic N-methyl-D-aspartate (NMDA) receptor. As in Huntington’s disease (HD), motor symptoms are linked to NMDA receptor-induced excitotoxicity in striatal medium spiny neurons (MSNs) of the indirect pathway, we propose that the same mechanism causing dyskinesia in LID.

Like hyperkinesia in HD (Sturrock and Leavitt, 2010), LID is also predominantly peripherally localized (Thanvi and Lo, 2004; Del Sorbo and Albanese, 2008). TD is characterized by both peripheral and orofacial dyskinesia, the latter being more prevalent in most patients. We have previously demonstrated that in PD patients the vulnerability to develop peripheral but not orofacial dyskinesia is associated with the presence of two variants of the GRIN2A gene (Ivanova et al., 2012). Moreover, we found that these genes were not associated with the likelihood of developing TD. We have demonstrated in the past that in TD orofacial dyskinesia is associated with other single nucleotide polymorphisms (SNPs) than peripheral dyskinesia (Al Hadithy et al., 2009; Al Hadithy et al., 2010). During the present study, we investigate whether this peripheral versus orofacial nature of LID in PD can be explained by considering SNPs for dopaminergic and serotonergic receptor genes, as a potential mechanism.

Materials and methods

Ethics Statement

The protocol was approved by the standing Institutional Review Board (Local Ethics Committee at the Siberian State Medical University, Tomsk, Russian Federation). Written informed consent was obtained from each patient. None of the participants had a compromised capacity/ability to consent; hence, obtaining consent from the next-of-kin was not necessary and not recommended by the Local Ethics Committee. The work described in this article was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, revised Fortaleza, Brazil, October 2013) for experiments involving humans.

Patients

The patients included in this study were retrieved from the Department of Neurology and Neurosurgery of the Siberian State Medical University (SSMU), Tomsk, Siberia, Russian Federation. Included were neurological patients who were suffering from dyskinesia and corresponding patients without dyskinesia having the same diagnosis (usually PD). In order to categorize patients accurately a clinical diagnosis protocol of PD was applied according to ICD-10 (G20) and the international clinical diagnostic criteria of the Parkinson’s UK Brain Bank of the PD Society of the United Kingdom (Hughes et al., 1992). The severity of Parkinson’s disease was determined by utilizing the Unified Parkinson’s Disease Rating Scale - UPDRS (Fahn et al., 1987) in the optimal “on” state. The stage of the disease was assessed using the “Hoehn and Yahr” scale (Hoehn and Jähr, 1967). The degree of movement disorders (hypokinesia, rigidity and resting tremor) was defined according to the section III of UPDRS scale, dedicated to movement disorders. The severity of dyskinesia was assessed according to the Abnormal Involuntary Movement Scale (AIMS) (Loonen and Van Praag, 2007). This scale consists of 12 items, of which 7 are basic indicators and are used for assessment of...
SNPs, or tag SNPs, that would accurately represent the majority of SNPs for 3 neurotransmitter genes: 10 SNPs for DRD3 gene (rs11721264, rs167770, rs3773678, rs963468, rs763291, rs2134655, rs9817063, rs324035, rs1800828, rs167771), 1 SNP for DRD4 gene (rs3758653), and 8 SNPs for HTR2C gene (rs6318, rs5946189, rs569959, rs17326429, rs4911871, rs3813929, rs1801412, rs12858300). The selection method was previously described by Xu and Taylor (freely available at http://www.niehs.nih.gov/snpinfo). We selected only tag SNPs that captured at least 10 SNPs.

### Statistical analysis

After having developed different strategies to account for missing data and interactions between different SNPs, classical logistic regression and a log-linear regression approach were used to analyze the data. The genotype prevalence was calculated separately in patients with and without dyskinesia to define the percentage of missing genotypes. The Hardy-Weinberg equilibrium test was applied with Fisher's exact test to groups. For the SNPs in the X-chromosomal HTR2C gene, deviation from HWE was not calculated. One SNP for HTR2C gene, rs12858300, was monomorphic; therefore, it was included analysis.

To analyze associations between the SNPs and the phenotypes, we used logistic regression for binary response traits and log-linear regression for continuous traits.

The following genetic models were tested:

1. Co-dominant; both alleles of a SNP influenced the phenotype
2. Dominant; rare allele homo- and heterozygotes were tested against common allele homozygotes.
3. Recessive; common allele homo- and heterozygotes were tested against rare allele homozygotes.
4. Over-dominant; heterozygotes were tested against both homozygote alleles.
5. Log-additive; a trend test for the genotypes, similar to the allele model, but comparisons were made among subjects (N) instead of chromosomes (2N). The test and estimates were based on a logistic regression model that coded the genotypes as 0, 1, or 2 to reflect the number of minor alleles.

The statistical significance of a SNP was established with a likelihood-ratio test that compared the effect of
a polymorphism with the null model. Age, sex, and
duration of disease were included into the models as
covariates. The Akaike information criterion was
applied to identify the model that best fits the data.
SNP effects were quantified with the odds ratio (OR)
and 95% confidence intervals. In the result tables, an
OR for a log-additive model corresponds to an
association between a rare allele and the presence of
dyskinesia. The ORs for other models correspond to
associations between the presence of dyskinesia and
rare allele homo- and heterozygotes (Dominant),
common allele homo- or heterozygotes (Recessive),
or heterozygotes (Over-dominant).

For the statistical analysis, we used both qualitative
traits (represented by the existence or absence of
dyskinesia) and quantitative traits (the severity of
dyskinesia represented by the sum of the AIMS
scores). Separately, the calculations for the total
dyskinesia and its subtypes: orofacial and limb-
truncal were done. Therefore, the following
phenotypes were analyzed.
1. Levodopa-induced dyskinesia (Schooler-Kane
criteria):
   a. Orofacial LID (AIMS 1-4);
   b. Limb-truncal LID (AIMS 5-7);
   c. Orofacial and limb-truncal LID (AIMS 1-7).
2. The severity of dyskinesia; total score for 1-4, 5-7,
   and 1-7.

Dyskinesia was considered a qualitative trait
(present/absent), but the degree of dyskinesia
expression was analyzed as a quantitative trait. To
avoid 0 values, 1 was added to the dyskinesia
expression values, and then they were log2-
transformed to obtain a log-normal distribution.

The statistical power was estimated based on the
prevalence of the tested phenotypes in the Siberian
sample and assumptions of a complete penetrance
and disease allele prevalence of 0.2-0.3. For orofacial
dyskinesia, to detect associations under different
genetic models with OR of 1.5, 2.0, and 2.5, the
power estimates varied between 14%-50%, 44%-94%,
and 78%-99%, respectively. For limb-truncal
dyskinesia, the power estimates varied between
10%-31%, 27%-75%, and 53%-95%, respectively.

For either type of dyskinesia, the power estimates
varied between 18%-65%, 58%-99%, and 91%-99%.
Overall, it would be fair to say, that our study was
reasonably powered to detect associations with OR,
as little as 2.0.

Results

First, the Hardy-Weinberg equilibrium test was
applied to analyze the frequency distribution of
investigated polymorphisms. One SNP of HTR2C
gene, rs12858300, was monomorphic; therefore, it
was removed from further analysis. The Hardy-
Weinberg equilibrium in patients was observed for the
following polymorphisms: rs3758653 (p = 0.894691),
rs2134655 (p = 0.562467), rs324035 (p = 1),
rs3773678 (p = 0.125774), rs167770 (p = 0.067768),
rs167771 (p = 0.397278), rs7633291 (p = 1),
rs1800828 (p = 0.795681). The Hardy-Weinberg
equilibrium was not calculated for polymorphic
variants rs6318, rs5946189, rs569959, rs17326429,
rs4911871, rs3813929, rs1801412, rs12858300 due
to the fact that the serotonin receptor gene HTR2C
is located on the X-chromosome.

The next step of the statistical analysis was
calculation of the best genetic models based on
Akaike information criterion (AIC); the log-additive
model is presented only in the case of statistical
significance. The correction for multiple comparisons
was not yet applied.

An association was found between presence or
absence of orofacial dyskinesia (according to the
Schooler-Kane criteria) and two investigated
polymorphisms in all 143 neurological patients:
rs3758653 (DRD4, Log-additive, Odds Ratio
\(OR = 2.31\) [95%CI=1.10-4.87], \(p=0.032\) and
rs4911871 (HTR2C, dominant, OR=2.88 [1.03 –
8.05], \(p=0.041\). These polymorphic variants, in
addition to a marker rs2134655 (DRD3, recessive
\(p=0.040\), are associated with dyskinesia and are
represented as a quantitative indicator according to
the sum of features from 1 to 4.

None of the studied polymorphisms was significantly
associated with qualitatively assessed limb-truncal
dyskinesia with the exception of rs963468 (DRD3,
dominant, \(p=0.034\) based on the quantitative
measures (table 1). Discovering this polymorphism
cannot help in calculating odds ratio, since all 11
patients with dyskinesia possessed G/G genotype.

When only the 101 patients suffering of Parkinson’s
disease (PD) are considered, the results are even
less convincing. Only rs3773678 (DRD3, dominant,
\(p=0.042\) was associated with orofacial dyskinesia.
No significant associations were found with respect to
limb-truncal dyskinesia or quantitative measures of
Discussion

The results were inconsistent between the extended (N = 143) and limited (N = 101) patient populations. The following methodological problems may explain these differences: multiple testing of DRD3 and HTR2C polymorphisms, diagnostic heterogeneity, and poor clinical diagnosis of dyskinesia in comparison to other movement disorders using the AIMS. However, we previously established a strong relationship with two polymorphisms of the GRIN2A gene and dyskinesia in the same patient population and with the same study design (Ivanova et al., 2012). In that study the association increased when we limited the extended population to the patients with PD. Therefore, it could be concluded that the currently described associations probably do not have a great clinical relevance.

A feature of our study is the separation of dyskinesia into two variants which phenotypically differ from each other and according to our hypothesis may have a different genetic background. The sum of the first four parameters on the scale was used for AIMS assessment of orofacial dyskinesia (these parameters include facial expressions and mouth area), the sum of 5-7 parameters correspond to the expression of limb-truncal dyskinesia (movement of the limbs and trunk); 1-7 items assess a total dyskinesia.

Table 1: Statistically significant associations between studied SNPs and phenotypes of dyskinesia in all neurological patients

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Orofacial LID</th>
<th>Limb-truncal LID</th>
<th>LID</th>
<th>1-4</th>
<th>5-7</th>
<th>1-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3758653</td>
<td>DRD4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4911871</td>
<td>HTR2C</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2134655</td>
<td>DRD3</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs963468</td>
<td>DRD3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

1-4 items represent the sum of the first four parameters on the AIMS used for assessment of orofacial dyskinesia (these parameters include facial expressions and mouth area); 5-7 items represent the sum of 5-7 parameters to the expression of limb-truncal dyskinesia (movement of the limbs and trunk); 1-7 items assess a total dyskinesia.

dyskinesia in this more limited, but pathologically more homogenous patient group.

Conclusion

In conclusion, the studied variants of dopamine and 5-hydroxytryptamine receptor genes may not contribute to the development of LID. However, they may be related to orofacial dyskinesia via a different mechanism. The exact role of these neurotransmitter receptors in the pathogenesis of the orofacial treatment...
dyskinesia remains to be elucidated.

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Conflict of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

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