Analysis of PITX3 Gene in Patients with Multisystem Atrophy, Progressive Supranuclear Palsy and Corticobasal Degeneration

Zygmunt Jamrozik¹, Mariusz Berdynski², Cezary Zekanowski², Anna Baranczyk-Kuzma³, Jarosław Sławek⁴, Magdalena Kuzma-Kozakiewicz¹, Aleksandra Maruszak², and Hubert Kwiecinski¹, ⁵

¹Department of Neurology, Medical University of Warsaw; ²Department of Neurodegenerative Disorders, Polish Academy of Sciences, Warsaw; ³Department of Biochemistry Medical University of Warsaw; ⁴Department of Neurology, Saint Adalbert Hospital, Gdańsk; ⁵Department of Neurological and Psychiatric Nursing, Medical University of Gdańsk, Poland

Abstract. Interactions of transcription factors Nurr1, Pitx3, and EN1 are involved in the maturation and survival of adult midbrain dopaminergic neurons during a lifetime. The aim of the study was to evaluate the presence of mutations and single nucleotide polymorphisms in PITX3 gene in clinically diagnosed multisystem atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD). In the group of 77 patients with MSA, 44 with PSP, and 6 with CBD, no pathogenic mutations were identified.

Key words: PITX3 gene-Multisystem atrophy- Progressive Supranuclear Palsy- Corticobasal Degeneration

Introduction

Maturation and survival of adult midbrain dopaminergic neurons during lifetime is dependent on interactions between nuclear receptor related protein 1 (Nurr1), homeobox protein engrailed-1 (EN1), and paired-like homeodomain transcription factor 3 (Pitx3) [1-4]. Nurr1 and Pitx3 are also involved in regulation of α-synuclein expression [5]. Pitx3 is essential in the final step of differentiation of dopaminergic neurons of the substantia nigra pars compacta. The loss of Pitx3 is lethal to neurons that should develop into this subpopulation of neurons. It also leads to generation of fewer neuronal projections to the caudate putamen and an altered morphology of ventral tegmental area neurons [6]. Mutations in the genes encoding Nurr1 and Pitx3 were found in few cases of Parkinson’s disease [7-9]. Degeneration of midbrain dopaminergic neurons in patients over 50 years of age and an accumulation of α-synuclein in oligodendroglia and neurons are present in multisystem atrophy (MSA) with parkinsonism or in clinical parkinsonian symptoms. Parkinsonism is also a prominent clinical feature of tauopathies, such as PSP and CBD. The aim of the study was to evaluate the presence of mutations or single nucleotide polymorphisms in PITX3 gene in clinically diagnosed MSA, PSP, and CBD cases.

Material and Methods

Seventy-seven patients with clinical diagnosis of MSA (45 with MSA type P and 32 with MSA type C), 44 with PSP, and 6 with CBD were included into the study (Table 1). All patients signed an informed consent prior to inclusion. DNA was isolated from peripheral blood leukocytes and, in 90 cases, from immortalized leucocytes using standard procedures [10]. The study was approved by Ethical Board Medical University of Warsaw; no KB/13/2006. Statistical analysis was performed using Fisher’s Exact Test.
Molecular analysis: the coding regions of PITX3 (exons 1-4) were amplified using intronic primers (sequences available on request). PCR products were purified with ExoSAP IT (USB) and sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The sequences were analyzed using the Sequencer software, version 4.10.1. Primers for PITX3:

Exon 1-F  5'- GTGGCAGTACGCGGTGAG -3'
Exon 1-R  5'- AAGGTCCAGCAATAGCTCCTC -3'
Exon 2-F  5'- GGACCTAGCTAAGCCGGAGA -3'
Exon 2-R  5'- TGGGGATGAAGCTGTTATGTC -3'
Exon 3-F  5'- GAGAATATGCGCTGGCTTG -3'
Exon 3-R  5'- GAAGGAGAGACGGTGTCA -3'
Exon 4-F  5'- CCGTCTCTAGCCACCTCATC -3'
Exon 4-R  5'- CCAGTCAAATGACCCCCAGT -3'
Exon 4-in  5'- TTTCATTCGGCTTCAACTC -3'

Results

No pathogenic mutations were identified in the entire coding region (exons 1-4) and in flanking intronic fragments of PITX3 in studied groups of PSP, MSA, and CBD patients. However, two single nucleotide polymorphisms (SNP) were detected. A novel polymorphic variant c.322T>G (G56G) was identified in exon 3 in a patient with a clinical diagnosis of PSP. To assess the possible pathogenic nature of the nucleotide substitution, the in silico analysis using ConSeq, ESEfinder 3.0, and NetGene2.4 was performed. It showed that c.322T position is highly variable in evolution, and that T>G substitution does not introduce novel splice signals. The second SNP identified in the reported group is a common variant rs2281983 (c.439C>T, I95I) with T allele frequency of 65%. No difference in alleles frequency for rs2281983 was found between MSA and PSP patients (Table 2).

Table 1. Characterization of the patients’ group

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number (female/male)</th>
<th>Age (years) mean (SD)</th>
<th>Age of onset mean (SD)</th>
<th>Clinical diagnosis probable/possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSA-P</td>
<td>45 (F 29/M 16)</td>
<td>61 (8.8)</td>
<td>57.8 (8.98)</td>
<td>36/9</td>
</tr>
<tr>
<td>MSA-C</td>
<td>32 (F 11/M 21)</td>
<td>59.7 (7.3)</td>
<td>55.3 (7.7)</td>
<td>28/4</td>
</tr>
<tr>
<td>PSP</td>
<td>44 (F 15/M 29)</td>
<td>67.5 (9.8)</td>
<td>63.7 (6.8)</td>
<td>40/4</td>
</tr>
<tr>
<td>CBD</td>
<td>6 (F 2/M 4)</td>
<td>63.8 (9.8)</td>
<td>61 (11.0)</td>
<td>3/3</td>
</tr>
</tbody>
</table>

MSA-P, multiple system atrophy-parkinsonism; MSA-C, multiple system atrophy-cerebellar; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; F, female; M, male.
It can be concluded that mutations in *PITX3* are not a common cause or a risk factor for MSA and PSP in the Polish population. Variant rs2281983 cannot be used in differential diagnosis of MSA and PSP. G56G substitution is most plausibly a silent and rare polymorphism.

**Acknowledgements**

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**References**


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**Table 2. Allele frequency of rs2281983 variant in MSA and PSP**

<table>
<thead>
<tr>
<th>rs2281983</th>
<th>MSA- P</th>
<th>MSA-C</th>
<th>MSA-P+C</th>
<th>PSP</th>
<th>P-value / Odd ratioa</th>
<th>P-value / Odd ratiob</th>
<th>P-value / Odd ratioe</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>7 (15%)</td>
<td>7 (22%)</td>
<td>14 (18%)</td>
<td>8 (18%)</td>
<td>P=0.7738 OR 0.7937</td>
<td>P=0.7836</td>
<td>P=1.000</td>
</tr>
<tr>
<td>CC vs. CT+TT</td>
<td></td>
<td></td>
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<tr>
<td>CT</td>
<td>21(47%)</td>
<td>8 (25%)</td>
<td>29 (38%)</td>
<td>14 (32%)</td>
<td>P=0.6124 OR 1.4</td>
<td>P=0.1942</td>
<td>P=0.5588</td>
</tr>
<tr>
<td>CT vs. CC+TT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TT</td>
<td>17 (38%)</td>
<td>17 (53%)</td>
<td>34 (44%)</td>
<td>22 (50%)</td>
<td>P=0.8199 OR 0.8824</td>
<td>P=0.2888</td>
<td>P=0.5734</td>
</tr>
<tr>
<td>TT vs. CC+CT</td>
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</table>

Two sided Fisher exact test

a/ PSP compared with MSA-C
b/ PSP compared with MSA-P
c/ PSP compared with MSA-P+MSA-C