Role of genetic polymorphisms of the dopaminergic system in Parkinson’s disease patients with impulse control disorders

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ABSTRACT

Background: The mechanisms underlying the development of impulse control disorders (ICDs) like compulsive gambling, buying, sexual, and eating behaviors in Parkinson’s disease (PD) are debated. We assessed whether allelic variants of dopamine D2 receptors (DRD2), catechol-O-methyltransferase (COMT) and dopamine transporter (DAT) were associated with the development of ICDs in PD.

Method: We enrolled 89 idiopathic PD patients (48 without ICDs and 41 with ICDs). All patients were screened with the Minnesota Impulsive Disorders Interview (MIDI) and fulfilled DSM-IV criteria for the ICD positive cohort. Differences in the frequency of the genotypes between ICDs and non-ICDs groups were assessed using the χ² test.

Results: Genotyping was performed for variants of the DRD2 Taq1A (rs1800497), COMT Val158Met (rs4680), DAT1 (3’ UTR 40 bp VNTR). Variants of DRD2 Taq1A, COMT and DAT1 were not associated with the risk of developing ICDs.

Conclusion: In our study, there were no differences in the frequency of variant of DRD2 Taq1A, COMT and DAT1 between the two groups. Polymorphisms of dopaminergic genes do not play a relevant role in the development of ICD in PD suggesting that ICD originate from inability to filter inappropriate behaviors triggered by dopaminergic therapy.

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1. Background

The mechanisms underlying the development of impulse control disorders (ICDs) like compulsive gambling, buying, sexual, and eating behaviors in Parkinson’s disease (PD) are debated. ICDs commonly develop after the initiation of dopaminergic therapy but predisposing behavioral traits have been recently reported also in newly diagnosed “de novo” patients [1]. Risk factors are use of dopamine agonists as well as novelty-seeking personality, impulsivity, and family history of alcohol use disorders [2]. Genetic polymorphisms of Dopamine D2 receptors (DRD2), dopamine transporter (DAT) and Catechol-O-methyltransferase (COMT) may contribute to the development of ICDs in PD through abnormal modulation of dopaminergic transmission and signaling in the mesocorticolimbic dopaminergic system [3].

DRD2 are most commonly found in GABAergic interneurons of the prefrontal cortex (PFC) and striatal regions. The human DRD2 gene contains several polymorphic sites and Taq1A locus located in the 3’ untranslated region of the gene. The DRD2 Taq1A restriction fragment length polymorphism (RFLP) results in an amino acid substitution (Glu713Lys) in a serine/threonine kinase which may affect substrate binding to the DRD2 receptor and it is associated with decreased striatal D2 receptors [4]. There is evidence for an association between DRD2 Taq1A polymorphism and alcohol dependence especially in impulsive form of alcoholism [12]. DRD2 Taq1A polymorphism was also associated with substance misuse dependence in both Caucasian and non-Caucasians. Furthermore, the DRD2 Taq1A polymorphism was related to pathological gambling suggesting a role in susceptibility to a range of maladaptive behaviors [13].

COMT is the major mammalian enzyme involved in the metabolic degradation of released dopamine (DA) and accounts for more than 60% of the metabolic degradation of DA in the prefrontal cortex. The Val158Met single nucleotide polymorphism is a common valine-to-methionine substitution, leading to a three to fourfold decrease
of COMT efficiency in Met/Met relative to Val/Val homozygotes, resulting in increased presynaptic dopamine concentration [5]. DAT plays a crucial role in determining the duration and amplitude of DA action by rapidly recapturing extracellular DA into presynaptic terminals after release. The DAT human gene displays a polymorphic 40 base pairs (bp) variable number of tandem repeat (VNTR). This polymorphism consists of a repetition of 40 bp that yields several alleles ranging from 3 to 11 repeats, alleles of 9 and 10-repeats being the most common [6]. Evidence suggests that the 9-repeat allele is associated with lower expression of the DAT, which may lead to slower DA clearance [7]. Some studies have found associations with COMT Val158Met, DAT VNTR with alcohol dependence and drug abuse [4].

In this study, we examined whether allelic variants of DRD2, COMT and DAT were associated with the development of ICDs in PD.

2. Methods

2.1. Subjects and data collection

In this case control study, PD patients were enrolled from the Parkinson’s disease Centre of the “San Camillo” Hospital (Venice Lido, Italy) and the Neurology Department at the University of Padua. We only included idiopathic PD patients diagnosed according to the United Kingdom Parkinson’s disease brain bank. ICDs were diagnosed according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders Text Revision criteria [8]. According to a previously published protocol [1], all patients were screened with the Minnesota Impulsive Disorders Interview (MIDI) and underwent a subsequent semi-structured interview which involved the caregiver (when present). Patients were recruited to obtain two DNA samples from each patient from which DNA was isolated from peripheral blood samples using QIAamp DNA Blood Mini Kit (QIAGen) and following the manufacture’s protocol. Genotyping of the following three polymorphisms was carried out: DRD2 Taq1A (rs1800497), COMT Val158Met (rs4680), DAT1 (3’ UTR 40 bp VNTR.

2.2. Molecular genetic analysis

DNA was isolated from peripheral blood samples using QIAamp DNA Blood Mini Kit (QIAGen) and following the manufacture’s protocol. Genotyping of the following three polymorphisms was carried out: DRD2 Taq1A (rs1800497), COMT Val158Met (rs4680), DAT1 (3’ UTR 40 bp VNTR.

2.3. PCR amplification

DAT1 VNTR was genotyped using a previously described method [10]. In brief PCR reaction was performed in 25 ul volumes using 60 ng of genomic DNA and contains 1× PCR buffer with 15 mM MgCl2 (QIAGen), 1× Q-Solution (QIAGen), 200 μM dNTPs, 0.5 μM of each primer and 1.5 units of HotStarTaq DNA Polymerase (QIAGen). Primers used were: D3 forward: 5’-GCT CAG GCC GCT CAT GCA GCA-3’ and D3 reverse: 5’-GAT GTG GCC ACC ACC TCA GAG AAA-3’. PCR cycles were at 94°C for 30 s, 65°C for 1 min, 72°C for 1 min; there was a 15 min preincubation step at 95°C and a 10 min 72°C after the completion of the cycles. PCR was performed in 2720 Thermal Cycler (Applied Biosystems). The PCR products were separated on 3% agarose gel stained with ethidium bromide. Genotyping results were confirmed by sequencing random samples.

2.4. High resolution melt (HRM) analysis

COMT Val158Met and DRD2 Taq1A were genotyping using High Resolution Melt (HRM) analysis. HRM-PCR was performed and monitored in CFX96 Real-Time PCR Detection System (Bio-Rad) and melting data were analyzed by Precision Melt Analysis Software (Bio-Rad). Primer used are: COMT forward: 5’-GAT CAA CCC CGA CTG TGC-3’; COMT reverse: 5’-TTC CAG GTC TGA CAA CGG-3’; DRD2 Taq1A forward: 5’-CAA CAC ACC CAT CCT CAA ACG-3’ and DRD2 Taq1A reverse: 5’-CC TCC CTA CTA GGA AGG AC-3’. We amplified DNA fragments from 20 ng of genomic DNA. Full reaction (20 μl) contains final concentration of reagents: 500 nM of each primer and 1× of SooFast EvaGreen Supermix (Bio-Rad). The amplification protocol consist of a first denaturation step at 98.0°C (2 min), 50 cycles of denaturation at 98.0°C for 2 s and annealing at 59°C for 5 s. Melting curves were generated by ramping from 65°C to 95°C with an increment of 0.2°C for 10 s. Genotyping results were confirmed by sequencing random samples.

2.5. Statistical analysis

The statistical analyses were conducted using the SPSS software (version 10.1.1, 2000). We used t-test for continuous variables and χ² test for categorical variables in comparing clinical characteristics between the groups. For the results of genotyping, deviations from Hardy-Weinberg equilibrium was tested using χ² test for which a P-value of 0.05 was considered significant. Differences in the frequency of the genotypes between ICDs and non-ICDs groups were assessed using the χ² test.

3. Results

A total of 89 PD patients was enrolled in the study, 41 classified as ICD positive (Table 1). ICD patients were younger and had earlier age at onset compared with control PD. There was trend for both higher M-dopa and dopamine agonist dose in ICD positive patients. There was also a trend for ICD to be more frequent in patients on COMT inhibitors (21 out of 41; 52% vs. 11 out of 48 in the ICD negative group).

The allelic frequencies for the three polymorphisms studied in PD with and without ICDs are shown in Table 2. The allele and genotype distributions of the three variants were all in Hardy-Weinberg equilibrium. Regarding COMT and DRD2 polymorphisms, homozygotes for the minor allele were grouped together with heterozygotes. There was no difference in frequencies of COMT Val158Met polymorphism in two groups χ² = 0.327, p = 0.568 in ICDs. The allelic distribution of the DRD2 Taq1A polymorphism did not reveal differences between the groups χ² = 0.937, p = 0.333.

The DAT1 VNTR grouping was based on the presence of the 9-repeat allele, resulting in 9-present (9+) and 9-absent (9−) groups. There were no differences in the frequencies of the 9+ and 9− in two groups χ² = 0.022, p = 0.882.

Table 1

<table>
<thead>
<tr>
<th>PD-ICD+</th>
<th>PD-ICD−</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>41</td>
<td>48</td>
</tr>
<tr>
<td>Male/Female</td>
<td>23/18</td>
<td>27/21</td>
</tr>
<tr>
<td>Age</td>
<td>61.39 (10.59)</td>
<td>68.58 (7.96)</td>
</tr>
<tr>
<td>Age of PD onset</td>
<td>52.68 (10.14)</td>
<td>57.33 (10.65)</td>
</tr>
<tr>
<td>Disease duration</td>
<td>5.00 (4.416)</td>
<td>11.44 (7.84)</td>
</tr>
<tr>
<td>Dopamine agonist</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Agonist LEDD,a mg/d</td>
<td>168.61 (114.92)</td>
<td>124.31 (113.94)</td>
</tr>
<tr>
<td>MAO-B</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>COMT inhibitors</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>LEDDb mg/d</td>
<td>728.48 (418.01)</td>
<td>581.67 (406.5)</td>
</tr>
<tr>
<td>Total LEDD©mg/d</td>
<td>897.09 (480.20)</td>
<td>705.98 (462.22)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>PD-ICD+</th>
<th>PD-ICD−</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMT</td>
<td>G/G</td>
<td></td>
</tr>
<tr>
<td>A carriers</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>DRD2 Taq1A</td>
<td>C/C</td>
<td>22</td>
</tr>
<tr>
<td>T carriers</td>
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<td>17</td>
</tr>
<tr>
<td>DAT1 VNTR</td>
<td>9-present</td>
<td>19</td>
</tr>
<tr>
<td>9-absent</td>
<td>22</td>
<td>29</td>
</tr>
</tbody>
</table>

Abbreviations: ICD = Impulse control disorder; LEDD = L-dopa equivalent daily dose; MAO-B = Monoamine oxidase inhibitors type B.

*a P-value is less than 0.05.

*b Agonist LEDD was calculated only from doses of dopamine agonist.

© Total LEDD was calculated as the doses of L-dopa plus the doses of dopamine agonists multiplied by theoretical equivalence.
4. Discussion

The development of ICDs in PD may be related to abnormal signaling and transmission in the dopaminergic pathway. In PD, excessive extra-synaptic dopamine, impaired reuptake of dopamine, or abnormal post-synaptic dopamine receptor stimulation and transmission by dopamine agonists may contribute to the development of these disturbances [11]. Evidence in favor of this hypothesis come from studies in alcohol dependence.

Two limiting factors of dopamine activity are the COMT and DAT which terminate dopamine activity by degradation and uptake, respectively. Genetic variants of COMT and DAT have been related to the enzymatic activity and protein availability. The Met allele of the COMT Val158Met polymorphism has been linked to lower enzymatic activity and several studies suggest that the 9-repeats allele of DAT 40 bp VNTR polymorphism, in comparison to the 10-repeats, is associated with lower levels of the transporter [6].

We found no association with the 9-repeat allele of DAT 40 bp VNTR polymorphism as well as the DRD2 Taq1A variant excluding a contribution of dopamine transporter and receptors. There was also no association between COMT Val158Met polymorphism and ICD development excluding a contribution of dopamine homeostasis.

In our study we considered only polymorphisms involved in the dopamine transmission as several reports did suggest that impulsivity is modulated primarily by dopamine. More importantly we used both the MIDI as well as a semi-structured interview to study the screen our population ensuring appropriate ICD diagnosis in all subjects [9]. We conclude that genetic polymorphisms of dopamine pathway are not related to development of ICD. Lack of significant medication dose differences between our two PD cohorts suggests that ICD may originate from inability of the basal ganglia to filter inappropriate behaviors once they are triggered by dopamine replacement therapy.

References
