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Tryptophan hydroxylase type 2 variants modulate severity and outcome of addictive behaviors in Parkinson's disease

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ABSTRACT

Background: Impulse control disorders and compulsive medication intake may occur in a minority of patients with Parkinson's disease (PD). We hypothesize that genetic polymorphisms associated with addiction in the general population may increase the risk for addictive behaviors also in PD.
Methods: Sixteen polymorphisms in candidate genes belonging to five neurotransmitter systems (dopaminergic, catecholaminergic, serotonergic, glutamatergic, opioidergic) and the BDNF were screened in 154 PD patients with addictive behaviors and 288 PD control subjects. Multivariate analysis investigated clinical and genetic predictors of outcome (remission *vs.* persistence/relapse) after 1 year and at the last follow-up (5.1 ± 2.5 years).
Results: Addictive behaviors were associated with tryptophan hydroxylase type 2 (TPH2) and dopamine transporter gene variants. A subsequent analysis within the group of cases showed a robust association between TPH2 genotype and the severity of addictive behaviors, which survived Bonferroni correction for multiple testing. At multivariate analysis, TPH2 genotype resulted the strongest predictor of no remission at the last follow-up (OR[95%CI], 7.4[3.27–16.78] and 13.2[3.89–44.98] in heterozygous and homozygous carriers, respectively, $p < 0.001$). The extent of medication dose reduction was not a predictor. TPH2 haplotype analysis confirmed the association with more severe symptoms and lower remission rates in the short- and the long-term ($p < 0.005$ for all analyses).
Conclusion: The serotonergic system is likely to be involved in the pathophysiology of addictive behaviors in PD, modulating the severity of symptoms and the rate of remission at follow-up. If confirmed in larger independent cohorts, TPH2 genotype may become a useful biomarker for the identification of at-risk individuals.

1. Introduction

Addictive behaviors in Parkinson's disease (PD), broadly including impulse control disorders (ICDs) and dopamine dysregulation syndrome (DDS), may complicate dopamine (DA) replacement therapy in up to 17% of patients [1]. ICDs and DDS have been increasingly conceptualized as "behavioral addictions", as they share several neurobiological features with substance use disorders [2–4]. In the general population, genetic factors contribute to addictive disorders in 30–60% of cases [5]. Similarly, personal and/or family history for ICDs or substance abuse is reported in up to 60% of PD patients with ICDs and DDS [1,6–8].

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Individual predisposition is likely to be related to the interaction among a number of gene variants, rather than to a single gene with Mendelian inheritance. As the pathophysiology of addictive behaviors in PD has not been fully elucidated yet, no specific and effective treatment is available. Current management strategies are essentially based on the reduction of DA-agonist therapy, but this approach is greatly limited by the worsening of motor fluctuations and the high risk of withdrawal symptoms [3]. These non-motor adverse events represent a remarkable distinctive feature compared to other complications of dopaminergic medications, such as the low remission rate in the long-term along with a high risk of relapse, even after discontinuation of DA-agonist therapy (estimated to be 36–45%) [9,10]. We believe that this feature represents another fundamental similarity supporting the conceptualization of ICDs and DDS in PD as addictive disorders and hypothesize that common genetic vulnerability factors are likely to be involved.

Here, we investigated a large cohort of PD patients using a *candidate-gene* approach aiming to identify polymorphic variants that increase the risk of developing ICDs by selecting gene polymorphisms previously associated with addiction in the non-PD population. In patients with addictive behaviors, we also investigated clinical and genetic predictors of outcome (remission *vs.* persistence/relapse) after 1 year and at the last follow-up.

2. Methods

2.1. Participants

Consecutive outpatients fulfilling UK Brain Bank criteria for PD, attending the Parkinson Institute (Milan, Italy) from April-2009 to April-2011 and contributing to the "Parkinson Institute Biobank" (www.parkinsonbiobank.com) were assessed for the presence of any addictive behavior (i.e. pathological gambling, binge eating, hypersexuality, compulsive shopping, internet addiction, and dopamine dysregulation syndrome). If any behavioral/psychiatric issue was found or suspected during routine neurological examination, patients underwent a 30-min semistructured interview by an experienced neuropsychologist (C.S) to confirm the diagnosis of pathological behaviors according to established criteria [1,2,6,8,10]. All patients were followed by the same neurologist for >12 months. The onset of pathological behaviors could have occurred also before 2009; in this case, we included only patients who had undergone a formal neuropsychological assessment at the onset of the disorder and had been continuously followed by the same neurologist over time. Caregivers were also independently interviewed, because patients may underestimate or deliberately deny pathological behaviors [e-1,e-2]. All PD patients and caregivers independently completed the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease-Rating Scale at the baseline assessment [e-3]. Behavioral outcome (dichotomized as remission *vs.* persistence/relapse) was recorded at two different time-lines of follow-up: one year (12 ± 4 months) after the onset of pathological behavior and the last follow-up visit available. Patients were considered "remitters" if they no longer met criteria for ICDs/DDS only if this was independently confirmed by the caregiver. As we believe that the definition of remission from addiction should include disappearance not only of the addictive behavior itself, but also of *craving*, we did not consider "remitters" those who would have persisted in their pathological behaviors if they had had the opportunity, but could not pursue their impulse only because of effective supervision (e.g. no longer gambling because of cash restrictions imposed by the caregiver/family members, who are the only guarantors of no relapse) [8,10]. Patients were considered non-remitters also in the event of at least one relapse during follow-up, after a variable period of remission. Exclusion criteria were: dementia according to the fourth edition text revised of the diagnostic and statistical manual of mental disorders [e-4] or Mini-Mental State Examination <26; neurological follow-up <6 months; suspected pathological behaviors and no formal neuropsychological evaluation; past behavioral abnormalities that were no longer present during the inclusion period. Additionally, we stratified the group of cases according to the following criteria: (a) *Severity of addictive behaviors*: QUIP score = 5/5 in one or more disorder and/or persistence of pathological behaviors according to diagnostic criteria despite discontinuation of the pharmacological trigger (DA-agonist therapy) for >4 weeks; (b) *Low dopaminergic therapy dose at onset of addictive behaviors*, defined as DA-agonist dose (in levodopa-equivalent daily dose, LEDD [e-5]) ≤150 mg/day and total LEDD (Levodopa + DA-agonist) ≤750 mg/day [11].

As control group, we used PD patients consecutively recruited from April-2010 to April-2011 who had no pathological behavior during the whole disease course despite being on DA-agonist therapy ≥300 mg LEDD for ≥6 months. Controls were similar to cases in terms of gender, age at PD onset, disease duration and dopaminergic medication daily dose. To minimize confounders, we excluded those reporting any behavioral change (i.e. QUIP score ≠ 0 in one or more items) even when it was below the pathological threshold defined by diagnostic criteria. All PD controls and their caregivers were interviewed independently.

The study was approved by the Ethics Committee. Informed consent was obtained according to the Declaration of Helsinki.

2.2. Molecular analysis

Among several biologically plausible candidate genes for which there is evidence of association with addiction and/or ICDs, we restricted our selection only to polymorphisms that were consistently replicated by ≥ 3 independent research groups in non-PD populations or ≥2 groups in PD, according to literature published up to June-2012. We selected 16 variants on 12 genes within five neurotransmitter systems and one neurotrophin (Supplementary Table S1).

Genomic DNA extraction was performed by standard methods (www.parkinsonbiobank.com). Single nucleotide polymorphisms (SNPs) were genotyped using the 5′ fluorogenic exonuclease assays (TaqMan), whereas variable number tandem repeats and insertion/deletion polymorphisms were genotyped by PCR assays followed by electrophoresis.

2.3. Statistical analysis

Statistical analyses were performed using the R program (http://www.r-project.org/), the PLINK v1.07 package, [e-6] or the CLUMP v2.4 software [e-7].

2.4. Genetic data

All 16 polymorphisms had a minimal overall call rate of 95%, an accuracy >99% (by random duplicated genotyping of 5% of the samples), and were tested for Hardy-Weinberg equilibrium (HWE) in control PD patients before inclusion in the analyses (PHWE>0.05). Deviations from HWE were tested using the exact test implemented in the PLINK software. For each polymorphism, a standard case-control analysis using allelic χ^2 test was used to provide asymptotic p-values, odds ratio (OR), and 95% confidence interval (CI), always referring to the minor allele. The most significant variants emerging from allelic analyses were subjected to the epistasis analysis and logistic regression models; in case of negative findings, we considered positive results at genotype analysis for

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