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Dopamine D3 receptor Ser9Gly variant is associated with impulse control disorders in Parkinson's disease patients

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ABSTRACT

Introduction: Impulse control disorders (ICD) are reported to occur at variable frequencies in different ethnic groups. Genetic vulnerability is suspected to underlie the individual risk for ICD. We investigated whether the allelic variants of dopamine (DRD3), glutamate (GRIN2B) and serotonin (HTR2A) receptors are linked to ICD in Indian Parkinson's disease (PD) patients.

Methods: We conducted a prospective, case-control study which included PD patients (70 with ICD, 100 without ICD categorized after direct psychiatric interview of patient and caregiver) and 285 healthy controls. Single nucleotide polymorphism (SNP) variants of *DRD3* p.S9G (rs6280), *GRIN2B* c.2664C>T (rs1806201) and *HTR2A* c.102T>C (rs6313) were genotyped.

Results: Multivariate regression analysis revealed that *DRD3* p.Ser9Gly (rs6280) heterozygous variant CT (OR = 2.22, 95% CI: 1.03–4.86, p = 0.041), higher daily Levodopa equivalent doses (LED) of drugs (for 100 mg LED, OR = 1.14, 95% CI: 1.01–1.29, p = 0.041), current dopamine agonist but not Levodopa use (OR = 2.16, 95% CI: 1.03–4.55, p = 0.042) and age of onset of motor symptoms under 50 years (OR 2.09, 95% CI: 1.05–4.18, p = 0.035) were independently associated with ICD.

Conclusion: *DRD3* p.Ser9Gly (rs6280) CT genotype is associated with ICD in Indian PD patients and this association is novel. Enhanced D3 receptor affinity due to gain-of-function conferred by the glycine residues could impair reward-risk assessment in the mesolimbic system and contribute to development of impulsive behaviour, in carriers of this genotype.

1. Introduction

Impulse control disorders (ICD) and related behaviours (ICRB) are a well recognized complication of dopaminergic treatment for Parkinson's disease (PD) with potentially devastating consequences [1,2]. Several hospital-based studies, using different survey methods, have reported a prevalence of ICD and related behaviours of 2–35%. [2,3]. Compulsive buying, eating, shopping and sexual behaviours are the common ICDs that occur significantly higher in PD patients than in healthy population [1]. Punding, hobbyism, walk-about and dopamine dysregulation syndrome are the other impulsive-compulsive behaviours reported in PD [1,3]. Several studies have shown that dopaminergic treatment, particularly dopamine agonist use, confers risk for the development of ICD, though PD by itself does not [4,5]. Not all PD patients develop ICD during treatment with dopamine agonists. Male gender, premorbid ICD, depression, current smoking and family history of addictive behaviours increase the ICD risk, pointing to an individual vulnerability that may be under genetic influence [1,2]. Genetic abnormalities in monoamine mediated neurotransmission are suspected to play a role in ICD. D3 dopamine receptor (*DRD3*) and NMDA glutamate receptor 2B subunit (*GRIN2B*) variants were associated with ICD in Korean PD patients while polymorphisms in *DRD1* and *2* and *GRIN2B* genes were associated with ICD in Malaysian PD

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patients [6,7]. These results have not been replicated in other populations.

The aim of this study was to evaluate the association of single nucleotide polymorphisms (SNP) *DRD3* p.S9G (rs6280), *GRIN2B* c.2664C>T (rs1806201) and *HTR2A* c.102T>C (rs6313) with the four common ICDs in Indian PD patients.

2. Methods

We reported earlier a cohort of 305 PD patients attending the movement disorder clinic of a University hospital, who were screened for the presence of ICD and subsequently, underwent direct, structured psychiatric interview for establishing the diagnosis [3]. In that cohort, patients whose treatment had been modified following the development of ICD were excluded as the study aimed to identify risk factors for ICD and ICRB [3]. For the present study, we recruited from this original cohort, all PD patients with one or more of the classical ICDs i.e., compulsive buying, eating, gambling and sexual behaviour (ICD+) and 100 PD patients without ICD (ICD-). Those subjects whose ICD resolved with treatment modification since the initial study, and new PD patients with a diagnosis of ICD, based on the same criteria, were included as ICD+ (regardless of the presence of active ICD at the time of blood draw) and the rest as ICD-as we were interested in analysing the ICD trait. As the pathogenesis of ICRB is considered to be different from the typical ICDs in PD, patients with only ICRB such as punding, dopamine dysregulation syndrome and hobbyism were not included. PD was diagnosed by a movement disorder specialist according to the United Kingdom Parkinson's Disease Society Brain Bank criteria [8]. Briefly, for the diagnosis of ICD or ICRB, all the patients were screened using the modified Minnesota Impulsive Disorders Interview (mMIDI) [9]. Those patients who screened positive were subjected to an elaborate test battery, which included the Diagnostic and Statistical Manual of Mental Health Disorders, Fourth Edition, Text Revision criteria (DSM IV-TR) for the diagnosis of pathological gambling and compulsive eating, the operational diagnostic criteria for hypersexuality and the McElroy's criteria for compulsive shopping [10–12]. ICDs were diagnosed based on a structured direct psychiatric interview of both the patient and the spouse/care giver, both independently and together. The evaluating psychiatrist was blinded to the treatment for PD. The clinical and demographic data, including the self reported ethnicity, the age at study, the age of onset of PD, the duration of dopamine replacement therapy, the current medications and their doses and the Hoehn and Yahr stage of the disease were also documented. All the assessments were done after taking the routine dose of dopaminergic medications, during the medication ON state. Scoring in the Unified Parkinson's Disease Rating Scale part III (UPDRS III) was also documented in the medication ON state [13]. The daily Levodopa equivalent dosage (LED) was calculated for all the dopaminergic medications using previously described formulae [14]. 285 healthy volunteers of the same ethnicity and who were not blood relatives of the patients were also recruited. All subjects provided written informed consent. The study was approved by the institutional ethics committee.

2.1. Genotyping (Table 1)

Peripheral blood was collected in K2EDTA BD Vacutainer® tubes and the genomic DNA was isolated using Qiagen Flexigene DNA kit as per manufacturer's protocol (Qiagen, Valencia, CA). The SNPs for *DRD3*, *HTR2A* and *GRIN2B* were selected based on the functional relevance and the minor allele frequency using genotype data obtained from Caucasian individuals in the HapMap project (HapMap Data Rel 24/Phase II Nov08, on NCBI B36 assembly, dbSNP b126). The primers flanking their respective SNPs were designed *in silico* using the PrimerZ software. *DRD3* p.Ser9Gly (rs6280) was screened using PCR RFLP while *GRIN2B* c.2664C>T (rs1806201) and *HTR2A* c.102T>C (rs6313) were screened using KASP™ assays (Table 1). The RFLP analysis of *DRD3* p.Ser9Gly (rs6280) was conducted by digesting the PCR products of 172 bp with *Msc*I restriction endonuclease (NEB, Inc., USA).

The genotyping of *HTR2A* and *GRIN2B* SNPs was conducted using KASP™ genotyping technology (LGC Genomics, UK) following the manufacturer's protocol. In brief, the Assay Mix was combined with Reaction Mix, containing the Taq polymerase enzyme, MgCl2, and the passive reference dye ROX. PCR was performed in a 96-well clear optical reaction plate in Applied Biosystems 7500 real time PCR machine (ABI, Foster city, CA) with SDS 7500 v2.0.5 software for absolute quantification. Duplicate samples were also included to ensure genotyping accuracy.

2.2. Statistical analysis

The genotype and allelic frequencies were computed and tested for deviation from Hardy-Weinberg equilibrium (ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The statistical comparison of means between the groups was performed using the student t-test and the Mann Whitney *U* test whenever necessary. The frequencies were compared using Chi square test and Fishers exact test. 3×2 chi-square tests were done initially for each gene to identify an association between frequency of the allelic variants and ICD status in PD. For the polymorphism found to have a significant association with ICD status, further 2×2 chi-squared tests were carried out to identify the genotype associated with ICD+/ICD-. Multiple logistic regression was used to predict ICD+ amongst the entire PD cohort (ICD+ and ICD-), with a model incorporating allelic variants and the clinically relevant variables whose univariate comparisons yielded a $p < 0.1$. Odds ratio (OR) are presented with their 95% confidence intervals (95% CI). P-value of <0.05 was considered as statistically significant. For an allelic variant with minor allele frequency 0.4–0.5 and with a 23% prevalence of ICD in PD, this study had 80% or more power to detect a 2.7-fold increase in the risk of ICD with recessive model (estimated by Quanto version 1.2.4., http://biostats.usc.edu/Quanto.html). The statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp., USA.

3. Results

3.1. Clinical characteristics

170 eligible patients (n = 70 ICD+ and n = 100 ICD-) and 285 healthy volunteers underwent genotyping and were included in the final analysis. All the patients were of Asian Indian ethnicity. At the time of inclusion in the study, the mean age of the PD patients was 57.2 ± 10.5 years. The mean age of disease onset was 49.1 ± 10.6 years and the average disease duration was 8.2 ± 5.0 years. The median of Hoehn and Yahr stage in the medication ON state was 2.0 (range 1.0–3.0) and in the medication OFF state was 3.0 (1.0–5.0). 81% of the entire cohort was on Levodopa and 58% were on dopamine agonists (Pramipexole or Ropinirole). The mean LED was 527.3 ± 327.6 mg/day.

The clinical characteristics of ICD+ and ICD- PD patients are shown in Table 2. Among the ICD+ PD patients, more than one ICD occurred in 11% (n = 8) of the patients. Compulsive buying was the most common ICD encountered in 49% (n = 34), followed by hypersexuality in 36% (n = 25), compulsive eating in 29% (n = 20) and pathological gambling in 9% (n = 6). Pramipexole (n = 46 in ICD +, n = 37 in ICD-) and Ropinirole (n = 11 in ICD +, n = 18 in ICD-) were