Genetic Variation Analysis in Parkinson Disease Patients With and Without Hallucinations

Case-Control Study

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Background: Visual hallucinations in Parkinson disease (PD) occur in approximately one third of patients treated long-term with dopaminergic medications. In Alzheimer disease, hallucinations and psychosis have been linked to increased representations of B2/B2 homozygotes for the dopamine receptor gene DRD1 and 1/1 or 2/2 homozygotes for DRD3. In addition, a previous study of PD patients with and without hallucinations did not show differences in D2 and D3 polymorphisms, although careful case-control matching was not performed. Another study linked the apolipoprotein E4 (APOE4) allele to hallucinations in PD.

Objective: To determine whether the frequency of dopamine receptor genetic variants and APOE alleles in patients with PD with and without chronic visual hallucinations resembles the pattern previously documented in patients with Alzheimer disease.

Methods: We conducted a case-control study of 44 patients with PD and chronic hallucinations and 44 patients with PD who had never hallucinated. Cases and controls were matched for current age and medications. DNA was isolated from blood samples and assayed for DRD1, DRD2, DRD3, DRD4, and APOE polymorphisms. Receptor polymorphisms were genotyped by polymerase chain reaction. Genotypes in hallucinators and non-hallucinators were compared using Mantel-Haenszel tests stratified by pair, and allele frequencies were compared using Wilcoxon signed rank tests within pairs.

Results: Neither D1 receptor genotypes (P = .37) nor allele frequencies (P = .38) differed, and there was no predominance of B2/B2 homozygotes in the hallucinators. For D3, there was a higher frequency of allele 2 (P = .047), but there was no significant difference between frequencies of homozygotes vs heterozygotes (P = .39) as reported in Alzheimer disease. D4 receptor distribution of long and short alleles did not differ between the 2 patient groups, and there were too few C alleles (3 of 86) to compare D2 allele genotypes or frequencies. For APOE, 12 cases and 12 controls carried E4 alleles (P > .99).

Conclusions: With careful case-control matching, visual hallucinations in PD are not associated with the pattern seen for patients with Alzheimer disease and visual hallucinations. Furthermore, there was no association between hallucinations and APOE. Similar methods using larger sample sizes might be adapted to test whether specific dopaminergic receptor genetic variants are associated with visual hallucinations in PD. Based on our data, the DRD3 allele 2 may merit further study.
PATIENTS AND METHODS

PATIENT SAMPLE

In our tertiary care PD treatment center, between February 1, 1999, and October 30, 1999, we identified all patients with PD, defined by CAPIT (Core Assessment Program for Intracerebral Transplantations) criteria,1 who were receiving dopaminergic therapy and had documentation of definite drug-related motor benefit. Patients with signs or a history suggesting Lewy body dementia, such as fluctuating cognitive function or neuroléptic sensitivity, were excluded. Using a questionnaire, we identified all white patients who experienced visual hallucinations at least 3 times weekly for the past 2 months and who had not experienced visual hallucinations before dopaminergic drug treatment. We chose only white patients because previous studies documented racial differences in dopamine receptor allele frequencies and because whites were the predominant racial group in our practice. All patients who fulfilled these entry criteria completed a Mini-Mental State Examination, a Parkinson’s Psychosis Rating Scale,8 a Unified Parkinson’s Disease Rating Scale (motor section), and Hoehn and Yahr stage evaluation and contributed a 3-mL blood sample for genetic testing. Of 354 patients screened, 48 met entry criteria, and 44 consented to participate. For each case, we used our computer database to identify potential control subjects with PD without hallucinations who were also white, fulfilled CAPIT criteria, and showed motor benefit from dopaminergic therapy. They were matched to individual cases for age (±3 years) and dopaminergic medications (levodopa, an agonist, or both). These subjects were interviewed at their next regular office visit and, if they had never hallucinated, were invited to participate as a matched control subject for a given case. Controls signed informed consent forms, completed the same tests, and contributed a blood sample for genetic testing. Of 98 controls identified by the computer search, 44 fulfilled entry criteria, and all consented. The research project was approved by the institutional review board of Rush University, Chicago, Ill.

SAMPLE SIZE AND POWER CONSIDERATIONS

Estimates of effect size and required sample sizes for detecting increased homozygosity of DRD1 and DRD3 receptor genes in patients with PD and chronic hallucinations were directly based on the work of Sweet et al. They documented a moderate effect size in detecting an increase in the DRD1 B2/B2 genotype and DRD3 homozygosity for either 1/1 or 2/2 (for both, $\chi^2=4.0$; $P<.05$; effect size, $w=0.30$ expressed as g [departure from equal probability] = 15) in individuals with AD and psychotic features compared with those with AD and no psychotic features.8 Assuming similar effect sizes in patients with PD, our sample size of 44 individuals in each group afforded 80% power to detect departures from equal probability of 15 points or larger for DRD1 B2/B2 and DRD3 1/1 or 2/2 homozygosity.

GENOTYPING

Genomic DNA was extracted from venous blood samples with a DNA isolation kit (Puregene; Gentra Systems, Minneapolis, Minn), and 100 ng was amplified by modifications of polymerase chain reaction and restriction protocols for genotyping DRD1, DRD2, DRD3, and DRD4 polymorphisms, as described elsewhere.10-13 Apolipoprotein E genotyping was performed via polymerase chain reaction amplification of 100 ng of genomic DNA followed by restriction analysis of radiolabeled products with CfoI, as published previously.16

STATISTICAL ANALYSIS

Data are presented as counts, means (SDs), or allele frequencies as appropriate. Cases and controls are compared using Mantel-Haenszel tests for genotypes of each receptor subtype and for APOE genotypes. DRD1 and DRD3 polymorphisms were analyzed by 3-category genotype and homozygosity. DRD4 genotypes were analyzed by comparing those with any long allele (6-8 repeats) with those with only short alleles. Apolipoprotein E polymorphisms were analyzed as exact genotype and by presence or absence of any APOE4 allele. Allele frequencies for D1, D3, and APOE within pairs were compared using Wilcoxon signed rank tests. Quantitative clinical characteristics of cases were compared with those of their matched controls using Wilcoxon rank sum tests.

RESULTS

CLINICAL DATA

The mean (SD) age of cases was 72.1 (8.4) years and of controls was 71.7 (7.9) years ($P=.11$). Cases and controls were matched for current dopaminergic drug exposure, with 12 pairs receiving levodopa alone, 1 pair receiving an agonist alone, and 31 pairs receiving combination levodopa and agonist treatment. Cases did not significantly differ from controls in mean (SD) daily doses of levodopa (655 [326] mg/d vs 613 [368] mg/d; $P=.20$) or agonist ( pergolide equivalent,3 2.37 [1.70] mg/d vs 2.24 [1.52] mg/d; $P=.48$). The mean (SD) PD duration was

not been extensively examined in patients with hallucinations, one study showed that the apolipoprotein E+ (APOE4) allele was associated with parkinsonian dementia in patients with nondemented PD. Because hallucinations and dementia can coexist, it is not clear whether the APOE marker in PD was a risk for hallucinations or later dementing illness. Based on these observations, we conducted a case-control study of patients with PD and hallucinations and a control group of patients with PD who never hallucinated to test (1) whether the pattern in AD is mimicked in patients with PD and hallucinations and (2) whether patients with PD and chronic hallucinations will have a significantly greater allele frequency of APOE4 than patients with PD who never hallucinated.
longer in cases (15.73 [7.0] years vs 10.25 [5.0] years; \(P<.001\)), but at the onset of chronic hallucinations in cases it was not significantly different from the current duration of PD in controls (12.20 [6.5] years vs 10.25 [5.0] years; \(P=.09\)).

Because item A of the Parkinson’s Psychosis Rating Scale measured the presence and severity of hallucinations, all controls scored 1 (absent) and all cases scored 2, 3, or 4 (mean [SD], 2.64 [0.60]). For the total of the remaining Parkinson’s Psychosis Rating Scale items, cases scored significantly higher than controls (mean [SD], 8.95 [2.42] vs 5.50 [0.63]; \(P<.001\)). The Mini-Mental State Examination scores of cases also were more impaired (mean [SD], 23.7 [5.3] vs 28.8 [2.4]; \(P<.001\)). Motorically, the 2 groups differed significantly, and the mean (SD) Unified Parkinson’s Disease Rating Scale motor score in cases was 38.5 (12.7) vs 28.2 (11.8) in controls (\(P<.001\)).

### DOPAMINE RECEPTOR AND APOE GENOTYPES

For DRD1, overall allele frequencies (0.31 for B1 and 0.69 for B2 alleles) and heterozygosity (0.39) were similar to previously reported \(^{1-10}\) values (B1 allele frequency range, 0.34-0.36; heterozygosity, 0.38-0.47) (**Table 1**). There was no significant difference in allele frequencies or in the distribution of genotypes between cases and controls, and, specifically, the B2/B2 genotype was not over-represented in the group with hallucinations. At the DRD2 locus, because only 3 individuals carried the C allele (2 cases and 1 control), no meaningful comparison of C allele frequency between the 2 study groups was possible. The overall frequency of the C allele variant was 0.017, similar to that reported in control groups from other studies \(^{1,11}\) (range, 0.018-0.03). For DRD3, overall allele frequencies (0.63 for allele 1 and 0.37 for allele 2) and heterozygosity (0.55) were similar to previously reported \(^{9,12}\) values (1 allele frequency range, 0.62-0.66; heterozygosity range, 0.4-0.49) (**Table 2**). There was a trend toward overrepresentation of the 2/2 genotype in cases (\(P=.05\)) but no significant difference in representation of the combined homozygote (1/1 and 2/2) genotypes between cases and controls (\(P=.45\)). Allele frequency analysis showed borderline increased frequency of the DRD3 2 allele in cases compared with controls (\(P=.047\)). This pattern did not reproduce the findings previously reported for AD. \(^{4}\) For DRD4, there was no difference in the percentage of individuals carrying at least 1 long allele between cases (45%) and controls (39%). However, the overall frequency of carrying at least 1 long allele was somewhat higher than reported in previous studies \(^{13,14}\) (range, 20%-31%). Allele frequencies (2 allele, 0.05; 3 allele, 0.07; 4 allele, 0.63; 5 allele, 0.02; 6 allele, 0.0; 7 allele, 0.23; and 8 allele, 0.005) at this locus showed a distribution similar to that in previous studies \(^{13,14}\) albeit with a slightly increased frequency of the 7-repeat allele.

For APOE, genotypes and allele frequencies were similar to those reported for whites from control populations living in the United States. \(^{16}\) There was no significant difference between cases and controls for the APOE genotype distribution, the fraction of individuals carrying an APOE4 allele (12 of 44 for both cases and controls), or the APOE4 allele frequency (**Table 3**). Furthermore, there was no association of the APOE4 allele with dementia, as defined by a Mini-Mental State Examination score of 24 or less.

### COMMENT

Hallucinations in PD are a frequent and clinically important problem in patients treated long-term with medications. Once hallucinations develop they persist, and their severity can progress to full psychotic behavior and agitation. \(^{17}\) Hallucinations are a significant risk factor for nursing home placement, and the latter is highly associated with subsequent death. \(^{18}\) These observations highlight the need for identifying patients at risk for hallucinations and the importance of developing effective therapies for hallucinations in this population.

Hypotheses concerning drug-induced hallucinations in PD have traditionally focused on the role of the dopaminergic system and, specifically, on dopaminergic hypersensitivity of mesocortical and mesolimbic D3 receptors. To date, however, we know of no pathological studies of dopaminergic receptor populations that have been performed in patients with PD and hallucinations.

Studies on genetic risk for hallucinations began with AD, another degenerative condition frequently associated with visual hallucinations and psychotic behavior. Psychosis was significantly more frequent in individuals expressing the B2/B2 homozygote pattern for DRD1. \(^{4}\) Likewise, psychosis was significantly more frequent in DRD3 homozygotes, either 1/1 or 2/2. Our primary aim was to test whether this same pattern occurred in patients with...
PD and hallucinations. The laboratory analytic techniques used for this documentation mirrored ours, but we were careful to match cases and controls for clinical features that have been posited to affect hallucinations, specifically, age and drug exposure. Our data demonstrate that the findings in AD do not directly extrapolate to PD and further suggest that hallucinations and psychosis in AD represent a distinct phenomenon from those in AD and may not be directly or solely related to dopaminergic mechanisms.

In focusing our study on the detection of similar patterns in hallucinating individuals with AD or PD, we built our power calculation and sample size determination directly on the previously cited study. Although the number of cases and controls is smaller than the group comparison study in patients with AD, the absence of any pattern of difference in the D1 receptor system leaves us confident that patterns of polymorphism in this receptor system do not mimic those seen in patients with AD and hallucinations. For the D3 system, we found a higher frequency of allele 2, but, again, the pattern seen in patients with AD did not occur.

A previous study examined D2 and D3 polymorphisms in patients with PD who did and did not have hallucinations and, like our study, found no group differences. The advantage of that study was its larger sample size of hallucinators and nonhallucinators with PD, and it attempted to focus on detecting polymorphism patterns distinctive to PD. It did not, however, examine the D1 or D4 systems. Our study complements and extends this study in several ways. Although our sample size was smaller, because we focused specifically on a comparison to the AD data already published, we included a detailed analysis of the D1 polymorphism. Another advantage of our study is that we carefully matched cases with controls in terms of demographic features that have previously been associated with hallucinations in PD, namely, age and medications. We also selected cases who were currently hallucinating so as to safely exclude those who may have transiently hallucinated in the past in the context of an acute infection or medication intoxication. Together, the 2 studies underscore a clear distinction between dopamine receptor polymorphisms in PD and those identified in patients with AD who hallucinate. Larger sample sizes will be required to establish possible associations of this D2 marker with hallucinations in PD because of the very low level of polymorphism at this locus.

Apolipoprotein E polymorphisms have been extensively studied in various neurodegenerative diseases as a result of the strong association of the APOE4 allele with AD. Whereas studies have not shown a relation between PD and APOE4 alleles, one study showed that in patients with PD and this allele, 76% had visual hallucinations compared with only 23% without the allele. This study did not specify details of the clinical material, and it is not clear whether the series was confounded with individuals who have Lewy body dementia, a parkinsonian syndrome characterized by hallucinations and an overrepresentation of the APOE4 allele. Our study did not confirm this finding, and we are confident that the clear clinical separation of cases and controls (frequent and chronic hallucinators vs patients who had never hallucinated), the long disease duration in both groups, and the absence of fluctuating cognition or neuroleptic sensitivity effectively eliminates Lewy body dementia as a confounding diagnosis. Our data on the overall allele frequency of APOE4 support previous findings that dementia in PD is not associated with an overrepresentation of APOE4.

Birkmayer and Riederer suggested that serotonergic/dopaminergic imbalance may be of primary importance in the pathogenesis of psychotic behavior in PD. Comparison of serotonergic receptor polymorphisms in cases and controls remains an area for future research. Because of the trend toward differences in the DRD3 genotype distribution between cases and controls and overrepresentation of the 2 allele in cases, a larger sample would be useful to determine whether the DRD3 2 allele is truly associated with hallucinations in PD. As we expand our study to the serotonergic system, we are collecting additional clinical pairs to enhance the analysis of this dopaminergic polymorphism.

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REFERENCES