Genetic Polymorphisms in Parkinson Disease Subjects With and Without Hallucinations

An Analysis of the Cholecystokinin System

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Background: Hallucinations in patients with Parkinson disease (PD), occurring in about one third of those receiving long-term dopaminergic therapy, contribute to morbidity and mortality. In matched Chinese PD subjects with and without hallucinations, the presence of the –45 C/T locus in the cholecystokinin (CCK) gene, particularly when combined with the CCK receptor, CCKAR (cholecystokinin A receptor), C polymorphism, was associated with increased hallucination risk. Because CCK gene polymorphisms vary across ethnic groups, the presence of similar associations in white PD subjects merits investigation.

Objective: To determine whether polymorphisms of CCK and CCK receptor genes are associated with hallucinations in white PD subjects.

Design: Case-control study of PD subjects with and without chronic hallucinations matched for age and dopaminergic medication. Genomic DNA was analyzed for CCK, CCKAR, and CCKBR (cholecystokinin B receptor) polymorphisms by polymerase chain reaction. Genotype distributions and allele frequencies were compared between groups and in matched pairs.

Results: Comparing matched pairs, we found more frequent representation of the CCK T allele in hallucinating PD subjects, although this finding was not statistically significant (P = .06). Of 5 cases with both CCK T and CCKAR C alleles, 4 were hallucinators. Cases and controls did not differ in CCKAR or CCKBR polymorphisms.

Conclusions: Our study supports a previous association of hallucinations in PD subjects with the CCK T allele and the combined CCK T and CCKAR C allele, suggesting that the CCK system may influence the development of hallucinations in PD subjects. The lower representation of the T allele in our white sample limited our statistical power. Further assessment of the T allele as a risk factor for hallucinations would include longitudinal study of nonhallucinators to detect the evolution of hallucinations relative to T allele frequency.

Arch Neurol. 2004;61:1280-1284

Various genetic polymorphisms have been investigated regarding association with increased risk of hallucinations in PD. One case-control study compared dopamine receptor and APOE (apolipoprotein E) polymorphisms in PD subjects with and without hallucinations. There was no significant difference in allele frequencies or distribution of genotypes between cases and controls for the dopamine receptor genes DRD1 and DRD3 or APOE4, although the DRD3 2 allele had a borderline increased frequency in hallucinators (P = .05). An important methodological strength of this study was the use of a case-control population matched for age and medications, factors often associated with hallucinations.

Cholecystokinin, a neuropeptide found in the gut and central nervous system, has
been implicated in dopaminergic regulation. The CCK and CCK receptor genes, CCKAR (cholecystokinin A receptor) and CCKBR (cholecystokinin B receptor), have been cloned and sequenced in humans. The CCK receptors are characterized by their different affinities for various forms of the CCK peptide, such as sulfated CCK8, unsulfated CCK8, and CCK4. Centrally, CCKAR mediates the behavioral actions of CCK and CCK-stimulated dopamine release in the posterior nucleus accumbens. In contrast, CCKBR mediates CCK inhibition of dopamine release in the anterior nucleus accumbens. The CCK gene thereby colocalizes in dopaminergic neurons in the central nervous system.

Because CCK modulates the dopamine system within the mesolimbic pathway, Fuji et al. compared CCK polymorphisms in PD subjects and healthy controls and found a significant difference in genotype distribution at the CCK –45 C/T locus between the 2 groups (P = .02; Bonferroni correction, P = .05). Furthermore, when comparing PD subjects with and without hallucinations, a significant difference was found at the CCK –45 C/T locus, with overrepresentation of CT and TT genotypes in hallucinators (P = .02; Bonferroni correction, P = .13). Wang et al. studied polymorphisms of CCK and its receptors, CCKAR and CCKBR, in PD subjects, with and without hallucinations, undertaking levodopa therapy. They found a difference in the genotype distribution at the CCK –45 C/T locus between the 2 groups (P = .003), with the T allele being overrepresented in hallucinators. In addition, the presence of a CCK T allele and a CCKAR C allele in combination significantly increased the risk of hallucinations in PD subjects (P = .01). Because allele frequencies vary across ethnic groups, we examined CCK polymorphisms in a similar size sample of white PD subjects.

**METHODS**

**PATIENT SAMPLE**

Subjects were initially selected from a database of idiopathic PD patients involved in ongoing longitudinal assessments of hallucinations at our tertiary care movement disorders center. Control subjects with PD and without prior hallucinations were drawn from the same physicians’ practices. This group was previously assessed for polymorphisms in the dopamine receptor gene and the APOE4 allele. Controls were matched for age within 3 years and dopaminergic medication (levodopa, agonist, or both) use. Only white subjects were studied. We collected data on demographic factors often associated with hallucinations in PD: MDS-UPDRS (Unified Parkinson’s Disease Rating Scale motor score), and PD duration, but to avoid overmatching, did not match cases and controls on these variables. The research project was approved by the institutional review board of Rush University, Chicago.

**SAMPLE SIZE AND POWER CONSIDERATIONS**

Because there are no published population studies on CCK genotype frequencies among white PD subjects, we based effect and sample size estimates on previous work with dopamine receptor polymorphisms in PD. Assuming similar effect sizes, a sample of 44 individuals in each group (case and control) afforded approximately 80% reasonable power. Estimates of effect size and required sample sizes for that study were previously established by Sweet et al.

**GENOTYPIC ANALYSIS**

DNA was isolated from 3-mL venous blood specimens with a DNA isolation kit (Puregene; Gentra Systems, Minneapolis, Minn). Coded DNA samples were saved in Tris-EDTA hydration buffer at 4°C before assay and at −80°C for long-term storage. Subjects underwent genotyping for CCK –45 C/T, CCKAR 779 T/C, and CCKBR 1550 G/A polymorphisms by polymerase chain reaction (PCR) and restriction analysis using previously described primer sets. Genotyping was performed on coded samples to ensure blinding with respect to cases and controls. The genotype of the CCK –45 C/T polymorphism was determined using the PfUMI restriction enzyme (New England BioLabs, Beverly, Mass), which digests the 158–base pair (bp) PCR product only when the T allele is present to give DNA fragments of 132 and 26 bp. The genotype of the CCKAR polymorphism was determined using the PsI1 restriction enzyme (New England BioLabs), which digests the PCR product only when the T allele is present to give DNA fragments of 264 and 480 bp. The genotype of the CCKBR polymorphism was determined using the HpyCH4 III restriction enzyme (previously known as Bst4ClI; New England BioLabs), which digests the 145-bp PCR product only when the T allele is present to give DNA fragments of 98 and 47 bp. The PCR mix contained the following: 1 µL of DNA, 0.3pM primers (Integrated DNA Technologies, Inc, Coralville, Iowa), 200µM nucleoside 5’-triphosphates, 0.25 U of DNA polymerase (Ampli Taq Gold; Applied Biosystems, Roche, Foster City, Calif), 2.5 µL of 10X PCR buffer (GeneAmp), and 2.5mM magnesium chloride. Samples were amplified using a PCR system (GeneAmp PCR System 9700; Perkin-Elmer, Wellesley, Mass) as follows: 12 minutes at 95°C, followed by 30 cycles of 95°C for 30 seconds, 38°C for 30 seconds, and 72°C for 60 seconds, and then 10 minutes at 72°C. The PCR products were incubated with 10× buffer and restriction enzymes at 37°C for 2 hours. After digestion with restriction enzymes, the products were subjected to electrophoresis on a 4% agarose gel (3:1) (Nu-Sieve; BioWhittaker Molecular Applications, Rockland, Me).

**STATISTICAL ANALYSIS**

Genotypes for the CCK gene and receptor subtypes were recorded for all subjects and analyzed for association with hallucinations. Genotype and sex frequencies in cases and controls were compared using Mantel-Haenszel tests. Two-sided sign tests were used to compare the difference in number of index alleles in cases and controls. The combination of the CCK T allele and the CCKAR C allele was analyzed by Mantel-Haenszel tests for synergistically increased risk of hallucinations in PD subjects. Statistical significance was at P < .05. Data are given as mean (SD) unless otherwise indicated.

**RESULTS**

**CLINICAL DATA**

The mix of cases and controls reflects the male predominance previously reported in PD subjects, but there were no significant sex differences between hallucinators and...
nonhallucinators (men, 55% [24/44] vs 61% [27/44]; P = .53).\textsuperscript{15,16} The age of the cases and controls was 72.1 (8.4) and 71.7 (7.9) years, respectively (P = .11). Dopaminergic treatment, as matched in cases and controls, yielded 12 pairs receiving levodopa alone, 1 pair receiving an agonist alone, and 31 pairs receiving levodopa and an agonist. Doses did not differ significantly between cases and controls taking levodopa (655 [326] vs 613 [368] mg/d; P = .20) and agonist (pergolide mesylate equivalent, 2.37 [1.70] vs 2.24 [1.52] mg/d; P = .48). In concert with prior publications on demographic features linked to hallucinations, PD duration was longer in cases than controls (15.73 [7.0] vs 10.25 [5.0] years; P = .09). Duration of disease at the onset of chronic hallucinations in cases, however, did not differ from the duration of PD without hallucinations in controls (12.20 [6.5] vs 10.25 [5.0] years; P = .90). Mini-Mental State Examination scores were also lower in cases than controls (23.7 [8.4] and 71.7 [7.9] years, respectively (P = .11). Dopa- 

**CCK AND CCKRECEPTOR POLYMORPHISMS**

Frequencies of CCK and CCKBR genotypes were in Hardy-Weinberg equilibrium. Low frequency of the CCKAR homozygous C genotype precluded adequate assessment for CCKAR association. For CCK, there was no significant (P = .10) difference in either genotype distributions or allele frequencies between cases and controls (Table 1). By measuring allele frequencies in matched pairs, however, we found that the CCK T allele was numerically over-

represented in PD subjects with hallucinations, although this difference did not quite reach statistical significance (P = .06).

For CCKAR and CCKBR, there were no significant differences in either genotype distribution or allele frequencies between cases and controls (Table 2). Although combined CCK and CCKBR and combined CCK and CCKAR genotypes did not reveal statistically significant (P = .17) differences between cases and controls, 4 of 5 cases with CCK T and CCKAR C alleles were hallucinators (Table 3).

**COMMENT**

In this study, we investigated the association of genetic polymorphisms of the CCK gene and its receptors with increased risk of hallucinations in PD subjects. Our study was based on a small but well-characterized matched case-control group of PD subjects with and without chronic hallucinations. We sought to replicate findings of Wang et al\textsuperscript{8} regarding the CCK system and to explore possible genetic differences in a white rather than a Chinese population. Although the low gene frequency among the cases and controls of white ethnicity precluded statistical significance, our study replicates patterns seen in prior studies suggesting that CCK polymorphisms may play a role in hallucinations in PD subjects. The overrepresentation of the CCK T in combination with the CCKAR C genotype among hallucinators suggests that together they may synergistically increase the risk of hallucinations.

We tested associations in our moderate-size sample by applying Mantel-Haenszel tests to compare cases with controls. To avoid decreasing our power, we were cautious about overmatching our cases and controls. We, therefore, matched on only 2 descriptive variables, age and current drug class exposure, because these features are generally used in clinical neurology to characterize groups of chronic PD subjects. When we compared the sex distribution of the groups, there was no significant difference, a finding concordant with a prior comparison\textsuperscript{17} of male and female PD subjects with hallucinations and delusions. Also, as reflected in prior studies,\textsuperscript{3,4} we found that hallucinations were associated

### Table 1. CCK Genotype Frequencies at -45 C/T*

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Allele</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C</td>
<td>70 (81)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>16 (19)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>14 (17)</td>
</tr>
<tr>
<td>Hallucinators</td>
<td>28</td>
<td>14</td>
</tr>
<tr>
<td>Nonhallucinators</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>22</td>
</tr>
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</table>

Table 2. CCK and CCK Receptor Allelic Frequencies

<table>
<thead>
<tr>
<th>Allele</th>
<th>CCK</th>
<th>CCKAR</th>
<th>CCKBR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hallucinators*</td>
<td>Nonhallucinators*</td>
<td>Total*</td>
<td>Allele Frequency</td>
</tr>
<tr>
<td>C</td>
<td>70 (81)</td>
<td>78 (91)</td>
<td>148 (86)</td>
<td>.14</td>
</tr>
<tr>
<td>T</td>
<td>16 (19)</td>
<td>8 (9)</td>
<td>24 (14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 (15)</td>
<td>11 (13)</td>
<td>24 (14)</td>
<td>.87</td>
</tr>
<tr>
<td></td>
<td>75 (85)</td>
<td>75 (87)</td>
<td>150 (86)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6 (7)</td>
<td>6 (7)</td>
<td>12 (7)</td>
<td>.80</td>
</tr>
<tr>
<td>G</td>
<td>82 (93)</td>
<td>80 (93)</td>
<td>162 (93)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CCK, cholecystokinin; CCKAR, cholecystokinin A receptor; CCKBR, cholecystokinin B receptor.
†The difference in the number of alleles is calculated as the count for the cases minus the count for the controls.
with lower cognitive function and more severe parkinsonism.

Ethnic differences in allele frequencies are important factors in studying genetic polymorphisms. Several previous studies on CCK receptor gene polymorphisms have concentrated on Asian populations. Compared with studies focusing on Asian populations, our white population had a lower representation of the CCK T allele. Although our sample size was similar to that of Wang et al, this racial genotypic difference limited our statistical power. We acknowledge the need for further recruitment of a larger population before subanalyses can be informative. Lower representation of CCK TC and TT genotypes among white subjects has been observed in other series as well, especially when compared with Chinese subjects. Cross-sectional prevalences of hallucinations have not included a large Asian representation, and most studies have focused on white subjects or have not designated the racial distribution of subjects. Even among white subjects, genetic polymorphisms may vary within subpopulations representing different ancestries.

In the central nervous system, CCK coexists with dopamine and regulates dopamine release in mesolimbic and mesocortical pathways. Cholecystokinin immunoreactivity in the substantia nigra has been reported as reduced in PD subjects. In a study by Fuji et al, a group of Japanese PD subjects with hallucinations compared with those without hallucinations had an increased frequency of the CCK –45 C/T polymorphism. Because the C to T transition was found in the Sp1-binding cis element of the CCK gene promoter, mutations at this location might affect promoter activity and gene transcription.

The CCK system has been studied in psychiatric disorders such as alcoholism, panic disorder, and schizophrenia with auditory hallucinations. Because hallucinations in PD subjects are predominantly visual, a direct comparison of CCK systems from other psychiatric conditions may be limited. Furthermore, the relationship of CCK and dopaminergic neuron degeneration and subsequent dopaminergic medication effects in PD subjects likely differ fundamentally from the models of alcoholism, anxiety, and schizophrenia.

Our study supports, but does not establish, a link between hallucinations in PD subjects and polymorphisms in the CCK system. We acknowledge the need for larger sample sizes to assess CCK and combined CCK and CCKAR effects, especially in racial groups with relatively low T allele frequency. In addition, prospective longitudinal studies of PD subjects focusing on the evolution of behavior complications may clarify the role of CCK and CCKAR polymorphisms as a risk factor for hallucinations. To extend the hypothesis that the CCK T allele, especially in combination with the CCKAR C allele, promotes the risk of visual hallucinations in PD subjects, the time to development of visual hallucinations in different genetic groups could be studied using survival analysis methods in a prospective design. In this case, we would hypothesize that PD subjects without the target genotypes would survive longer without developing hallucinations than PD subjects carrying the CCK T and CCKAR C profile.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Hallucinators (n = 43)</th>
<th>Nonhallucinators (n = 43)</th>
<th>Total (N = 86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK CC and CCKAR TT</td>
<td>22 (51)</td>
<td>26 (60)</td>
<td>48 (56)</td>
</tr>
<tr>
<td>CCK CC and CCKAR CT or TT</td>
<td>6 (14)</td>
<td>9 (21)</td>
<td>15 (17)</td>
</tr>
<tr>
<td>CCK CT or TT and CCKAR TT</td>
<td>11 (26)</td>
<td>7 (16)</td>
<td>18 (21)</td>
</tr>
<tr>
<td>CCK CT or TT and CCKAR CT</td>
<td>4 (9)</td>
<td>1 (2)</td>
<td>5 (6)</td>
</tr>
</tbody>
</table>

Abbreviations: See Table 2.
*Data are given as number (percentage) of each group.

Accepted for Publication: December 2, 2003.

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Author Contributions: Study concept and design: Goetz and Berry-Kravis. Acquisition of data: Goldman, Goetz, Berry-Kravis, and Zhou. Analysis and interpretation of data: Goldman, Goetz, Berry-Kravis, and Leurgans. Drafting of the manuscript: Goldman, Goetz, Berry-Kravis, and Zhou. Critical revision of the manuscript for important intellectual content: Goldman, Goetz, Berry-Kravis, and Leurgans. Statistical expertise: Leurgans. Obtained funding: Goetz. Administrative, technical, and material support: Goldman and Zhou. Study supervision: Goetz and Berry-Kravis.

Funding/Support: This study was supported by the Parkinson’s Disease Foundation, New York, NY.

REFERENCES


**Correction**

**Error in Author Name.** In the article titled “Neuropsychologic Status in Multiple Sclerosis After Treatment With Glatiramer,” published in the March issue of the ARCHIVES (1999;56:319-324), the author names should have appeared as follows: Amy Weinstein, PhD; Steven R. Schwid, MD; Randolph B. Schiffer, MD; Michael P. McDermott, PhD; Daniel W. Giang, MD; Andrew D. Goodman, MD. The ARCHIVES regret the error.