Preprohypocretin Polymorphisms in Parkinson Disease Patients Reporting "Sleep Attacks"

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Study Objectives: Previously, we found a significant association between the dopamine D2 receptor gene polymorphism Taq IA and sudden onset of sleep in patients with Parkinson disease. Here we evaluated the association between the preprohypocretin (-909T/C), (-22C/T), and (-20C/A) polymorphisms and sudden onset of sleep in the same population of patients with Parkinson disease.

Design: We conducted an association study analyzing the distribution of preprohypocretin polymorphisms in German, caucasian Parkinson disease patients with and without sudden onset of sleep, matched according to drug therapy, disease duration, sex, and age.

Setting: Movement disorders section at a university hospital.

Participants: 132 Parkinson disease patients with sudden onset of sleep and 132 Parkinson disease patients without sudden onset of sleep.

Interventions: Blood samples were taken from each participant and used for DNA extraction. Polymorphisms were analyzed by established polymerase chain reaction protocols or direct sequencing.

Measurements and Results: The variant allele T of the (-909T/C) preprohypocretin polymorphism was more commonly found in Parkinson disease patients with sudden onset of sleep. Statistical analysis showed that there were significant differences in the genotype (P = .024) and allele (P = .018) distribution between both groups. For heterozygous and homozygous carriers of allele T, the genotype relative-risk estimates for the presence of sudden onset of sleep were 2.01 (95% confidence interval: 0.76-5.34) and 2.81 (95% confidence interval: 1.09-7.25), respectively.

Conclusions: Our results show a significant association between the (-909T/C) preprohypocretin polymorphism and sudden onset of sleep in Parkinson disease. However, we could not demonstrate any interaction between the Taq IA and (-909T/C) polymorphisms with respect to the occurrence of sudden onset of sleep, suggesting that multiple genetic factors may contribute to the pathogenesis of this phenomenon.

Keywords: Dopamine receptor, polymorphism, sudden onset of sleep, Parkinson disease, narcolepsy, preprohypocretin, hypocretin (HCRT), orexin.

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INTRODUCTION

SLEEP DISORDERS AND DAYTIME SLEEPINESS ARE FREQUENT FINDINGS IN PATIENTS WITH PARKINSON DISEASE (PD).2,6 THE UNDERLYING CAUSES ARE STILL CONTROVERSIAL. IN 1999, “SLEEP ATTACKS” (TERMED SUDDEN ONSET OF SLEEP [SOS] IN THIS PAPER) WERE DESCRIBED IN PD PATIENTS TAKING THE NONERGOLINE DOPAMINE-RECEPTOR AGONISTS PRAMIPEXOLE AND ROPINIROLE.7 SOS IS DEFINED BY ABRUPT EPISODES OF UNPLANNED SLEEP DURING ACTIVITIES OF DAILY LIVING IN WHICH THEY ARE NOT EXPECTED TO OCCUR (EG, SPEAKING, EATING, DRINKING, STANDING, COOKING, AND DRIVING). SINCE NONERGOLINE DOPAMINE-RECEPTOR AGONISTS HAVE A NEGligible AFFINITY TO THE DOPAMINE D2-RECEPTOR FAMILY (IE, DRD1 AND DRD5), WE HYPOTHESIZED THAT SOS MAY BE ASSOCIATED WITH AN ALTERATION OF DOPAMINERGIC TRANSMISSION INVOLVING A MEMBER OF THE DOPAMINE D2-RECEPTOR FAMILY.8,9 THEREFORE, WE PERFORMED A STUDY INVESTIGATING THE DISTRIBUTION OF PREVIOUSLY IDENTIFIED POLYMORPHISMS IN THE FAMILY OF DOPAMINE D2-RECEPTOR GENES (DRD2, DRD3, DRD4) IN PD PATIENTS WITH AND WITHOUT SUDDEN ONSET OF SLEEP, DAYTIME SLEEPINESS, OR BOTH SUDDEN ONSET OF SLEEP AND DAYTIME SLEEPINESS. WE COULD SHOW A SIGNIFICANT ASSOCIATION BETWEEN THE DRD2 RECEPTOR GENE POLYMORPHISM TQA IA AND SOS IN PD.1

Narcolepsy is characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis and usually begins during adolescence.9 Hypocretins play a major role in the pathophysiology of narcolepsy.10 Human narcolepsy is probably caused by deficient hypocretin neurotransmission in the lateral hypothalamus.11 Animal models have shown narcolepsy syndromes to be caused by mutations in the hypocretinergic system.12 In humans, hypocretin-1 is almost undetectable in the cerebrospinal fluid (CSF),10 and the pathogenesis of narcolepsy appears to be multifactorial and triggered by genetic and environmental factors. Neuropathologic studies have demonstrated an almost complete loss of hypocretin mRNA and peptides in human narcoleptic brains.13 A possible link between the dopaminergic and hypocretinergic system with respect to sleep-wake regulation has been suggested by the recent observations that the catechol-O-methyltransferase (COMT) genotype is significantly associated with daytime sleepiness in patients with PD and narcolepsy.4,11,12 We therefore hypothesized that the hypocretin system may also be involved in the pathogenesis of SOS in PD and investigated the distribution of the previously described preprohypocretin (-909T/C), (-22C/T), and (-20C/A) polymorphisms16,17 in PD patients with and without SOS. Hypocretin-1 and hypocretin-2 are neuropeptides processed from a common precursor, preprohypocretin.10

Disclosure Statement

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PATIENTS AND METHODS

Patients

In 2000, a questionnaire on SOS in PD was sent to 12,000 members of the German Parkinson Association (Deutsche Parkinson-Vereinigung), the national patient support group.18 The methods and results of this survey have been presented elsewhere.18 We used the data from this survey to collect blood samples from PD patients with (group A) and without (group B) SOS, matched according to drug therapy, disease duration, sex, and age.1 Patients were defined as having SOS if they reported unexpected SOS at least 2 to 4 times per month and a subjectively abrupt transition from wakefulness to sleep. With the data set that we used, it was possible to obtain a near-to-perfect matching concerning the criteria type and dosage of dopamine-receptor agonist and levodopa dosage. The patients were recruited by a semistructured telephone interview in which the diagnosis of PD was reviewed by questions concerning the disease symptoms and levodopa responsiveness. Subsequently, the patients’ general practitioner collected the blood samples and sent them to our laboratory. Previously, we investigated the allele and genotype frequencies of several polymorphisms in the DRD2, DRD3, and DRD4 genes in this population.1 Now 264 (n = 132 in each group) of these PD patients receiving a combination therapy with levodopa, dopamine-receptor agonist, and other antiparkinsonian drugs were analyzed with respect to the role of the hypocretin system in the pathogenesis of SOS. All patients were of Germanic, caucasian origin. The study was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was given by all participants.

Genotyping

Genomic DNA was isolated from venous blood samples and subjected to polymerase chain reactions followed by restriction enzyme digestion using standard laboratory protocols.19,20 The allele frequency and genotypic distribution of the (-909T/C), the (-22C/T), and the (-20C/A) polymorphisms in the preprohypocretin gene were determined as described elsewhere.17

Statistical Analysis

First we tested whether our populations were in Hardy-Weinberg equilibrium. Comparison of genotype and allele frequencies between the groups was subsequently carried out using Cochran-Armitage trend tests and Pearson χ² tests, respectively. A P value of .05 was considered significant. Furthermore, genotype relative risks and predictive values were estimated for the (-909T/C) polymorphism. Besides, we descriptively compared the expected and observed genotype frequencies of the Taq IA polymorphism in the DRD2 gene and the (-909 T/C) polymorphism in the preprohypocretin gene. The statistical analyses were performed using the programs SAS (SAS Institute, Cary, NC) and SPSS 11 (SPSS, Inc., Chicago, Ill).

RESULTS

Table 1 shows descriptive statistics of both groups. Ninety-four percent of all patients were treated with levodopa. The average daily dosages for levodopa (362.0 mg [group A] versus 371.5 mg [group B]), pramipexole (1.14 mg vs 1.12 mg), ropinirole (4.0 mg vs 4.4 mg), bromocriptine (12.9 mg vs 12.8 mg), pergolide (2.24 mg vs 2.30 mg), α-dihydroergocryptine (41.5 mg vs 40.0 mg), cabergoline (4.63 mg vs 4.44 mg), and lisuride (0.70 mg vs 0.77 mg) were almost identical. Further antiparkinsonian medications included anticholinergic agents (group A: 50 cases; group B: 58 cases), N-methyl-D-asparate inhibitors (group A: 47 cases; group B: 49 cases), COMT-inhibitors (group A: 20 cases; group B: 17 cases), and others (group A: 39 cases; group B: 27 cases).

The genotype distribution in patients with SOS and controls did not differ from Hardy-Weinberg equilibrium. Table 2 shows the distribution of the (-909T/C) preprohypocretin polymorphism alleles and genotypes in the 2 groups. The variant allele T of the (-909T/C) polymorphism was more commonly found in PD patients with SOS as compared with PD patients without SOS. Statistical analysis showed that there were significant differences in the genotype (P = .024) and allele (P = .018) distribution between PD patients with SOS and the control group. For heterozygous and homozygous carriers of allele T, the genotype relative-risk estimates for the presence of SOS were 2.01 (95% confidence interval [CI]: 0.76-5.34) and 2.81 (95% CI: 1.09-7.25), respectively. The positive predictive value of allele T carriers was 0.52 (95% CI: 0.45-0.58), and the respective negative predictive value was 0.70 (95% CI: 0.47-0.87).

We also performed direct sequencing analysis of the hypocretin promoter/exon-1 region in 264 PD patients. In the present study, we did not find the (-22C/T) polymorphism in any PD pa...
tient with SOS, but we did find it in 1 patient without SOS. Furthermore, we did not identify any patient featuring the (-20C/A) variant in our population.

Table 3 shows the comparison between the expected and observed genotype frequencies of the Taq IA polymorphism in the DRD2 gene and the (-909T/C) polymorphism in the preprohypocretin gene. Using descriptive statistics, the expected and observed genotype combination frequencies of these 2 polymorphisms were quite similar.

**DISCUSSION**

**Hypocretin and PD**

The current study shows that there is a statistically significant association between the (-909T/C) preprohypocretin variant allele T and PD patients with SOS. The (-909T/C) polymorphism is a common C-to-T variant located within an Alu repeat region approximately 3 kb upstream of the start codon.17

The hypocretin-signaling system is important in the regulation of the sleep-wake cycle and is disturbed in narcolepsy.18 Narcoleptic patients suffer from symptoms such as excessive daytime sleepiness and shortened latency of sleep. The role of hypocretin in PD, however, remains controversial. Compared to narcolepsy, PD has been suggested to be associated with normal CSF levels of hypocretin.21 Furthermore, 3 patients with early-stage PD, who reported excessive daytime sleepiness during dopamine-receptor agonist treatment, had normal hypocretin-1 values in the CSF.22 In another study, hypocretin-1 concentrations in ventricular CSF in 19 parkinsonian patients were measured and compared with those of neurologic controls, such as patients with essential tremor and severe dyskinesia secondary to a midbrain stroke or multiple sclerosis.23 In patients with PD, hypocretin levels were negatively correlated with disease severity, as measured by the Unified Parkinson Disease Rating Scale in an off-treatment period. Hypothalamic cellular loss is usual in PD, with the lateral and posterior nuclei being the main targets of degenerative process in this area.24 Accordingly, hypocretin levels in the CSF may reflect the size of the hypocretin neuron pool,25 and a decrease in hypocretin levels may indicate degeneration of hypocretin neurons in PD. The greater decrease in HCRT levels in the CSF with increasing disease severity suggests progressive hypocretin neuron degeneration. The authors of this study proposed that hypocretin deficiency may occur mainly at the advanced stage of PD, as suggested by the negative correlation between hypocretin levels and motor disability.23

**Hypocretin Polymorphisms**

Polymorphisms in the promoter region of hormone genes have been implicated in disease susceptibility, most notably in the HLA-associated autoimmune disorder type 1 insulin-dependent diabetes mellitus, in which a variable number of tandem-repeat polymorphisms upstream of the preproinsulin gene are known to be associated with increased insulin-dependent diabetes mellitus susceptibility.26 Peyron et al13 have suggested in previous work that the 2 hypocretin-receptor genes are not major contributors for narcolepsy susceptibility, based on the observation that frequent hypocretin receptor-1 and hypocretin receptor-2 polymorphisms were not associated with this disorder. Furthermore Hungs et al17 reported that the (-909T/C) preprohypocretin polymorphism was not associated with human narcolepsy using randomly selected narcolepsy patients, most of whom were HLA-DQB1*0602 associated and without a family history. Gencik et al16 identified a (-22C/T) polymorphism in exon-1 of the hypocretin gene in 6 of 178 narcolepsy patients versus 1 of 189 controls and postulated a significant association. This finding was not confirmed by Hungs et al.17 Another study examined the same sequence in 72 narcoleptic subjects versus 24 control subjects. In this study, a single-base-pair change at position -20 (C to A) was observed in a single patient without HLA-DQB1*0602.13 This polymorphism was observed in a few unaffected Caucasian control subjects, supporting its likely benign nature.17 The effect of these polymorphisms on the phenotype, eg, the degree of daytime sleepiness in narcolepsy patients, has not yet been addressed.

**Dopamine, Hypocretin, and Daytime Sleepiness**

The DRD2 gene is located on chromosome 11q22-q23. Most studies of the Taq IA polymorphism are related to alcohol and other substance abuse.27-29 Wieczorek et al30 screened gene regions for association with narcolepsy by a microsatellite-based approach, describing their finding that the most frequent allele 5 of the DRD2 microsatellite was significantly increased in the patient group. Animal and postmortem studies in humans have shown that the dopaminergic system is disturbed in narcolepsy, with special emphasis on the cataplectic aspect of the disorder.31 Recently, Eisensehr et al32 reported that the D2-receptor binding was elevated in narcolepsy and correlated positively with the frequency of cataplectic and sleep attacks. The authors emphasized that the human striatal dopaminergic system is altered in vivo in narcolepsy with cataplexy. Additionally, Dauvilliers et al14 found a strong effect of a functional polymorphism of COMT activity on disease severity in narcolepsy. COMT is one of the major enzymes in dopamine metabolism, and different activities of this enzyme could potentially result in different rates of turnover of endogenous dopamine or dopamine derived from exogenous sources, such as levodopa, in subjects with different COMT genotypes.15 The finding that COMT activity is important for the disease severity in narcolepsy makes an involvement of COMT in the pathogenesis of “sleep attacks” in PD conceivable. Frauscher et al15 demonstrated an association between a COMT genotype and subjective daytime sleepiness in PD patients. The authors speculated that the mechanism of action of COMT on daytime sleepiness might involve a higher availability of endogenous or exogenous dopamine at sleepiness-related brain structures, with dopaminergic binding sites such as the ventral tegmental area or the mesostriatal system.31 Hypocretin neurons have widespread

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projections, with dense excitatory projections to all monoaminergic and cholinergic cell groups. The hypocretin transmission is higher in the active period and is activated by sleep deprivation. It has been suggested that hypocretin neurons drive monoaminergic activity to control sleep-wake states. Reports of sleep disturbances in dopaminergic disorders such as PD emphasize dopamine’s role in sleep regulation. Sleep can be controlled by D1 or D2 presynaptic receptor modulation of the ventral tegmental area but not by the activity of the substantia nigra. Dopaminergic neurons of the ventral tegmental area, but not the substantia nigra, are excited by hypocretin. Additionally, the existence of sleep-state dependent dopaminergic neurons in the ventral periaqueductal gray has been demonstrated. These findings suggest that the dopaminergic neurons in the ventral tegmental area and ventral periaqueductal gray, which are under the control of hypocretin neurons, may be important in regulating wakefulness. In the present work, we did not identify any PD patient with SOS featuring the (-22C/T) or (-20C/A) preprohypocretin polymorphism. This result suggests that these variants do not contribute significantly to the pathogenesis of “sleep attacks” in PD. Since we previously found a significant association between the DRD2 Taq IA polymorphism and SOS in PD, we analyzed the distribution of the expected and observed (-909T/C) and DRD2 Taq IA genotypes in patients with and without SOS. Since these values were similar, we suggest that—despite the proposed interaction of the dopaminergic and hypocretinergic system in the regulation of daytime sleepiness—the (-909T/C) variant allele T and the DRD2 Taq IA variant allele A2 contribute as independent risk factors to the occurrence of “sleep attacks” in PD. This result indicates that multiple genetic factors play a role in the pathogenesis of this still poorly recognized phenomenon.

**SUMMARY**

The current study suggests that the T allele of the (-909T/C) preprohypocretin variant is associated with a significantly increased risk of developing SOS in PD patients. The T allele is likely not useful as a predictor of SOS in the routine clinical management of PD patients, although C/C homozygotes may be less susceptible to the development of SOS. Further studies are mandatory to confirm this single association in independent samples.

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