Sex Differences in Clinical and Genetic Determinants of Levodopa Peak-Dose Dyskinesias in Parkinson Disease

An Exploratory Study

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Background: Several factors, both clinical and genetic, may account for the risk of developing levodopa-induced peak-dose dyskinesias (PDD) in patients with Parkinson disease, but it is unclear how these factors interact for modulating the individual susceptibility for PDD.

Objective: To examine clinical and genetic risk factors for determining individual susceptibility of PDD in patients with Parkinson disease.

Design: Cohort study.

Setting: Referral center for Parkinson disease in Calabria, southern Italy.

Patients: Two hundred fifty patients with Parkinson disease were screened for the presence or absence of PDD following a short-term levodopa administration, and 215 subjects were available for further evaluations, including genotypic analysis of the CA dinucleotide short tandem repeat (CAn-STR) polymorphism located in the dopamine receptor D2 gene (DRD2).

Results: One hundred five patients (48.8%) exhibited PDD following short-term levodopa administration, and 110 patients (51.2%) did not. Multivariate logistic regression analysis showed that independent predictors for the occurrence of PDD were female sex, earlier age at onset of Parkinson disease, longer duration of treatment, and higher dose of levodopa. Genetic factors related to the DRD2 CAn-STR polymorphism were not independent predictors for PDD in the total population, but they had a strong protective effect on the appearance of PDD when the multivariate analysis was performed in men (odds ratio, 0.34 [95% confidence interval, 0.14-0.84]). In women, a genetic protective effect on PDD was not evident.

Conclusions: Risk factors for PDD, both clinical and genetic, act in different ways for men and women. Genetic factors related to the DRD2 polymorphic status have a protective effect on PDD development in men but not in women. A female sex-related effect for the risk of PDD may be so strong that it overcomes any protective effect due to genetic factors.

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EVODOPA-INDUCED DYSKINESIAS are frequent and disabling complications observed during long-term treatment of patients with Parkinson disease (PD). There is no clear consensus regarding the frequency of dyskinesias, but a recent analysis of previously published levodopa treatment series indicated that patients treated with levodopa for 4 to 6 years have approximately a 40% likelihood of experiencing dyskinesias.1 The development of dyskinesias has been reported to depend on several clinical risk factors, such as earlier age at onset of PD,2 longer duration of disease,3 longer duration of levodopa treatment,4 higher levodopa dose,2,5 and female sex.6-8 Nevertheless, these clinical variables may only partially account for the risk of developing dyskinesias, and a substantial proportion of patients with PD never develop dyskinesias. Genetic factors could contribute to the individual variations in the development of dyskinesias, and we have previously reported that certain alleles of an intronic polymorphism of the dopamine receptor D2 (DRD2) gene reduced the risk for the appearance of the most common type of levodopa-induced dyskinesias (ie, peak-dose dyskinesias [PDD]).9 To date, however, the relationship between clinical and genetic factors in determining the risk for the development of PDD during levodopa therapy is still unknown. None-
thless, recognition of such a relationship may help to clarify the mechanisms underlying individual susceptibility for PDD.

To identify putative risk factors for determining individual susceptibility of PDD, we examined the effect of several clinical and genetic variables on the occurrence of PDD in a cohort of patients with PD.

**METHODS**

**PATIENTS**

Two hundred fifty patients with PD participated in this study. They were consecutively selected starting from January 1999 until June 2002 among the outpatients of the Institute of Neurology of the University “Magna Graecia” of Catanzaro, Italy. Inclusion criteria were (1) clinical diagnosis of PD according to the United Kingdom Parkinson’s Disease Society Brain Bank criteria; (2) duration of levodopa treatment of a minimum of 6 months; (3) presence or absence of PDD observed following a short-term levodopa administration, excluding patients with other types of levodopa-induced dyskinasias, such as biphasic dyskinasias and off-dose dystonia; and (4) no family history of PD extended to the first-degree relatives. According to these criteria, 27 patients were excluded from the study for the presence of biphasic dyskinasias (n=8), off-dose dystonia (n=7), or family history of PD (n=12). Another 8 patients refused to participate in the study. Therefore, 213 patients (123 men and 92 women; mean age±SD, 66.7±8.7 years) were included in the present study. All patients were white, born in Calabria (southern Italy), and gave a fully informed consent for the study.

Peak-dose dyskinasias were recorded as present or absent by the examining neurologist on the occasion of a short-term oral levodopa test. Briefly, the levodopa test consisted of a 6-hour observation by a trained neurologist after the morning administration of a standard dose of 250 mg of levodopa plus 25 mg of carbidopa after a night not taking any drug and in a fasting state. Clinical status was assessed by means of the Unified Parkinson’s Disease Rating Scale at baseline and at 30, 60, 90, 120, 240, and 360 minutes after levodopa administration. The following clinical characteristics were available for all the patients: age, sex, weight, severity of the disease measured by the modified Hoehn-Yahr scale, age at onset of the disease (defined as the first diagnosis made by a neurologist), duration of disease, duration of levodopa therapy, and daily levodopa dose.

**MOLECULAR GENETIC ANALYSIS**

Genotypic analysis of the intronic CA dinucleotide short tandem repeat (CAₙ-STR) polymorphism, located in intron 2 of the DRD2 gene (1.25 kilobase upstream of exon 3), was performed on all patients. Genomic DNA was prepared from leukocytes harvested from whole blood with standard methods, and genotyping of the CAₙ-STR polymorphism was performed as described. All genotyping was performed by individuals who were unaware of the clinical status of the subjects.

**STATISTICS**

Statistical analysis was performed by considering the patients as classified into 2 groups depending on the presence or absence of PDD. The clinical variables were compared between the groups by means of the 2-sample, unpaired t test for continuous variables with approximate normal distribution, the Mann-Whitney U test for nonparametric variables, and the χ² test for discrete variables. Genotype and allele frequencies were compared between the groups using crosstabulation and the χ² statistic, under the assumption of the Hardy-Weinberg equilibrium. To assess the contribution of each individual variable on the appearance of PDD, univariate and multivariate logistic regressions were performed. The latter, a forward stepwise procedure, was used to select the best set of independent predictors, with a criterion for entry of P<.25; only the variables with P<.05 were kept in the model. The relative risk was estimated through calculation of odds ratios with 95% confidence intervals.

**RESULTS**

Of the 215 examined patients, 105 (48.8%) exhibited PDD and 110 (51.2%) did not. Only 8 patients with observed PDD on the levodopa test did not report dyskinesias in their medical history, whereas all the patients without observed PDD did not historically report dyskinesias. Thus, the positive predictive value of PDD observed on the levodopa test with regard to the historically reported dyskinesias was 92%, and the negative predictive value was 100%. Clinical details of patients with dyskinetic and nondyskinetic PD are given in Table 1. Subjects with dyskinesias were younger, had a female-sex preponderance, and weighed less than patients without dyskinesias; moreover, patients with PDD were more disabled as measured by the Hoehn-Yahr scale, had an earlier age at onset of PD and a longer duration of disease, were treated for a longer period with levodopa, and took a larger daily levodopa dose than patients without PDD. Univariate logistic regression analysis showed that age, female sex, body weight, Hoehn-Yahr score, age at onset of PD, duration of the disease, duration of levodopa treatment, and levodopa dose were predictors for the appearance of PDD.

In the CAₙ-STR polymorphism, 4 alleles (characterized by the presence of 13, 14, 15, or 16 CA repeats) and 10 resulting genotypes were identified. The observed genotype frequencies did not differ from the expected frequencies according to the Hardy-Weinberg equilibrium (P = .08). Because of the low frequencies of some genotypes, subjects were categorized as 13,14+ or 13,14− (ie, subjects carrying at least 1 of the 13 or 14 alleles in their genotype or subjects carrying neither the 13 nor the 14 allele, respectively). In the total sample of examined patients (Table 2), the genotypic distribution of the CAₙ-STR polymorphism was significantly different between patients with and without dyskinesias, and subjects carrying the 13,14+ genotype had a decreased risk for the appearance of PDD. However, when the comparisons were performed by considering the sex of subjects with and without dyskinesias, only men carrying the 13,14+ genotype had a decreased risk for PDD, whereas in women the genotypic status of the CAₙ-STR polymorphism was similar between subjects with and without dyskinesias and did not influence the risk for PDD. The multivariate logistic regression analysis was performed including clinical and genetic variables in the statistical model. The best set of independent predictors differed when considering the total sample or by analyzing
Indeed, in the total sample, independent predictors for the occurrence of PDD were female sex, age at onset of PD, duration of treatment, and levodopa dose; for men, the best set of independent predictors for PDD included age at onset of PD, H14067 genotype carrier status, levodopa dose, and body weight; and for women, the only variable included in the model was age at onset of PD.

**COMMENT**

This study highlights the role of several clinical and genetic factors in determining the occurrence of levodopa-induced PDD in patients with PD. To our knowledge, this is the first study attempting to investigate well-known clinical risk factors for PDD together with factors involved in genetic susceptibility for PDD. Our findings indicate that risk factors for PDD, both clinical and genetic, act in different ways for men and women. Indeed, we found that in men independent predictors for the occurrence of PDD were an earlier age at onset of PD, a peculiar polymorphic status in the DRD2 gene, a larger daily dose of levodopa, and a body weight less than 61 kg, whereas in women only an earlier age at onset of PD best predicted the occurrence of PDD. These data suggest a sex-related effect on clinical and genetic risk factors associated with the development of PDD.

Individual susceptibility to certain conditions, such as PDD, may depend on the imbalance between factors that increase or decrease the risk for that condition. In our study, univariate analysis showed that many clinical variables were associated with an increased risk for PDD, whereas the examined genetic factors provided evidence that some genotypes were protective against the development of PDD. However, some of the tested clinical variables were related (age, age at onset, severity of PD, disease duration, treatment duration, and levodopa dose) and their effect on the occurrence of PDD vanished or decreased when they were entered into the multivariate logistic regression model. Overall, in our total population, the variables remaining in the model were female sex, earlier age at onset, longer duration of treatment, and higher dose of levodopa. The most significant risk factor for PDD in multivariate analysis was female sex.
In women, a genetic protective effect on PDD was not evident. However, women had a 3-fold increased risk for PDD than men, and this sex-related effect could be so strong as to overcome any protective effect due to genetic factors. On the other hand, other factors, such as those due to levodopa exposure, did not enter into the multivariate model for women, thus suggesting that female sex mostly accounted for the risk of PDD and strongly influenced other variables. This sex effect on PDD may warrant further studies on other variables related to the female sex, such as the factors involved in the reproductive function or in the genotypic status of genes coding for hormone receptors.

Our findings, however, should be considered preliminary because there are a number of limitations to the present study. First, the investigated sample was a referral-based cohort, and as such, it may not be representative of the overall population of patients with PD. Second, it is uncertain whether experimentally provoked and observed dyskinesias following a short-term levodopa test, such as in this study, are a good surrogate marker for dyskinesias that occur in patients receiving long-term treatment; nevertheless, serial monitoring of the motor response for several hours following administration of a standard levodopa dose is typically informative, allowing discrimination of PDD from other types of dyskinesias, and in our sample, there was a good concordance between experimentally observed and historically reported dyskinesias. Third, there are many other genetic factors (ie, genes involved in dopaminergic transmission or other systems underlying the control of movement) and other nongenetic variables (eg, drugs, hormones, substances such as coffee) that could influence the occurrence of PDD and have not been investigated in the present study. Nevertheless, our study, even with these limitations, has practical implications. Recognizing peculiar profiles of risk for PDD may aid in improving strategies to prevent these troublesome complications. For instance, drugs inducing fewer dyskinesias, such as dopamine agonists, could be administered to subjects at greater risk, whereas levodopa could be safely given to those individuals who are at lower risk for PDD, mainly men with a later onset of PD and carrying specific genetic DRD2 polymorphic variants. Further studies are needed to confirm these issues.

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