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ABSTRACT: Objectives: The objective of this study was to determine phenotypic features that differentiate nonparkinsonian first-degree relatives of PD leucine-rich repeat kinase 2 (LRRK2) G2019S multiplex families, regardless of carrier status, from healthy controls because nonparkinsonian individuals in multiplex families seem to share a propensity to present neurological features.

Methods: We included nonparkinsonian first-degree relatives of LRRK2 G2019S familial PD cases and unrelated healthy controls participating in established multiplex family LRRK2 cohorts. Study participants underwent neurologic assessment including cognitive screening, olfaction testing, and questionnaires for daytime sleepiness, depression, and anxiety. We used a multiple logistic regression model with backward variable selection, validated with bootstrap resampling, to establish the best combination of motor and nonmotor features that differentiates nonparkinsonian first-degree relatives of LRRK2 G2019S familial PD cases from unrelated healthy controls.

Results: We included 142 nonparkinsonian family members and 172 unrelated healthy controls. The combination of past or current symptoms of anxiety (adjusted odds ratio, 4.16; 95% confidence interval, 2.01-8.63), less daytime sleepiness (adjusted odds ratio 1 unit, 0.90; 95% confidence interval, 0.83-0.97), and worse motor UPDRS score (adjusted odds ratio 1 unit, 1.4; 95% confidence interval, 1.20-1.67) distinguished nonparkinsonian family members, regardless of LRRK2 G2019S mutation status, from unrelated healthy controls. The model accuracy was good (area under the curve = 79.3%).

Conclusions: A set of motor and nonmotor features distinguishes first-degree relatives of LRRK2 G2019S familial PD cases from unrelated healthy controls.
probands, regardless of mutation status, from unrelated healthy controls. Environmental or non-LRRK2 genetic factors in LRRK2-associated PD may influence penetrance of the LRRK2 G2019S mutation. The relationship of these features to actual PD risk requires longitudinal observation of LRRK2 familial PD cohorts. © 2018 International Parkinson and Movement Disorder Society

**Key Words:** Parkinson’s disease; G2019S; LRRK2; motor UPDRS; anxiety; daytime sleepiness

The leucine-rich repeat kinase 2 (LRRK2) G2019S is a causative mutation for Parkinson’s disease (PD) with a reduced, and variable age-related penetrance that is found in approximately 5% to 6% of familial PD cases and 1% to 2% of sporadic cases. Additional non-LRRK2 genetic or environmental factors may contribute to a higher penetrance in multiplex families when compared with bona fide isolated PD cases. In a prior case-control LRRK2 G2019S mutation family study, LRRK2 mutation-carrying nonparkinsonian first-degree relatives of LRRK2 G2019S PD cases were different from unrelated healthy controls on several clinical phenotypic variables and shared several of these differences with their non-mutation-carrying first-degree relatives: higher motor UPDRS (mUPDRS) scores, a higher incidence of symptoms of constipation, and worse color discrimination. In another LRRK2 family study comparing nonparkinsonian relatives and controls, olfaction was worse in nonparkinsonian relatives not carrying a LRRK2 G2019S mutation than those with a mutation. The generalizability of this finding remains to be demonstrated though, because another study did not find differences between three similar groups. Overall, the clustering of motor and nonmotor signs in LRRK2 families independent of the corresponding mutation suggests that other genetic modifiers and environmental factors play a role in an increased risk for parkinsonism in these families, as recently reported for the Dynamin-3 gene. The rarer event of parkinsonian phenocopies in LRRK2 families (LRRK2 G2019S mutation negative) is consistent with this hypothesis. Examples drawn from other forms of familial forms of PD provide further support to this hypothesis: in a family with PTEN-induced kinase–associated PD, hyper-echogenicity of the substantia nigra was present in nonparkinsonian family members when compared with controls, regardless of the PTEN-induced kinase mutation status.

Consequently, we postulate that the existence of more homogeneous phenotypic groups with a predisposition to neurological dysfunction in nonparkinsonian family members of LRRK2 G2019S mutation multiplex families may help in the discovery of genetic or environmental factors that put LRRK2 G2019S mutation carriers in these families at risk of PD. As a first step, we identified motor and nonmotor features in nonparkinsonian patients belonging to LRRK2 G2019S mutation multiplex families using a large sample of unrelated healthy participants as controls.

**Methods**

**Study Design**

This was a secondary analysis of a multicenter cross-sectional family study. Participating centers were (1) Toronto Western Hospital Movement Disorders Centre, Toronto, Canada; (2) the Parkinson’s Institute and Clinical Center, Sunnyvale, California, USA; (3) the Institute of Neurogenetics, Lübeck, Germany; and (4) the Parkinson’s Disease and Movement Disorders Unit, Hospital Clinic de Barcelona, Spain.

**Population**

The participants were part of 2 previously reported LRRK2 cohorts with data collected between 2006 and 2009 and 2011 to 2015, respectively. Both original LRRK2 cohorts had ethics committee approval.

For the present study, we included (1) nonparkinsonian, first-degree relatives of LRRK2-associated PD cases, with and without LRRK2 G2019S mutations, and (2) nonparkinsonian unrelated controls defined as individuals without a neurologic disease recruited among nonblood relatives of the patients with idiopathic PD and volunteers identified through advertisements in study centers (Lübeck, Germany; Toronto, Canada; Barcelona, Spain).

**Analysis**

We selected a priori nonmotor and motor features considered to be early motor symptoms of PD and/or prodromal nonmotor features. Variables related to motor phenotype were the total score of the “old” mUPDRS (continuous factor), item 4 on the postural/action upper extremity tremor, and the following items of the UPDRS part II related to motor performance: difficulty with handwriting (item 8), difficulty turning in bed (item 12), and difficulty with walking (item 15). These items were treated as binary variables: absence (score 0) versus presence (score 1-4). The variables associated with nonmotor features were individual questions about prior or current symptoms of depression, anxiety, or constipation, and the item of intellectual impairment (item 1) of
the UPDRS part I collected as binary variables (presence vs absence). We considered as continuous variables the MMSE, the Beck Depression Inventory-II, State-Trait Anxiety Inventory form Y, Epworth Sleepiness Scale, and the Brief Smell Identification Test. The Barcelona cohort used the MoCA, the University of Pennsylvania Smell Identification Test, and the Hospital Anxiety and Depression Scale-Anxiety scale. The MoCA and University of Pennsylvania Smell Identification Test scores were converted to MMSE and Brief Smell Identification Test scores, respectively, using published algorithms.\(^{11,12}\) When the “new” MDS-UPDRS\(^ {13}\) was used, the scores were converted to the “old” mUPDRS\(^ {10}\) as described before in one of the original cohorts.\(^ 3\) We used score standardization to statistical distribution for the State-Trait Anxiety Inventory and Hospital Anxiety and Depression Scale-Anxiety scale scores.

**Statistical Analyses**

**Univariate Analyses.** Using a logistic regression model, we tested the association of each of the variables described previously with membership to nonparkinsonian relative LRRK2 G2019S familial cases versus an unrelated healthy control group.

**Multivariate Analysis and Variable Selection.** Variables meeting the threshold of \( P \leq 0.25 \) after univariate analyses were included in an initial model. We used a backward variable selection method to obtain the final model, excluding independent variables with an associated \( P > 0.1 \). Age and gender were forced into the model, as biological confounders. Model validation was done using bootstrap resampling (500 replications) to test the stability of estimates.\(^ {14}\) The model results were presented as odds ratio and corresponding 95% confidence intervals. All analyses were performed using Stata/IC 14 (StataCorp, College Station, Texas).

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Adjusted OR (95% CI)</th>
<th>Test statistic, ( Z ) value</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at evaluation (1-year increment)</td>
<td>0.94 (0.92-0.96)</td>
<td>-5.21</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Gender (female vs male)</td>
<td>0.94 (0.54-1.62)</td>
<td>-0.24</td>
<td>.814</td>
</tr>
<tr>
<td>ESS score (1-unit increment)</td>
<td>0.90 (0.83-0.97)</td>
<td>-2.74</td>
<td>.006</td>
</tr>
<tr>
<td>Presence of past or current symptoms of anxiety (presence vs absence)</td>
<td>4.16 (2.01-8.63)</td>
<td>3.83</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>mUPDRS (1-unit increment)</td>
<td>1.41 (1.20-1.67)</td>
<td>4.07</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

We used a multiple logistic regression model followed by backward variable selection. ESS, Epworth Sleepiness Scale; mUPDRS, motor section of the Unified Parkinson’s Disease Rating Scale. Hosmer-Lemeshow test: \( P > .413 \).
We included 142 LRRK2 first-degree relatives and 172 unrelated healthy controls, all nonparkinsonian. Among the LRRK2 G2019S mutation family members there was no difference between mutation carriers and noncarriers for the variables considered (Table 1). In an unadjusted univariate comparison, the group of nonparkinsonian first-degree relatives (regardless of a LRRK2 G2019S mutation status) had higher mUPDRS scores, (mean [standard deviation, SD]: 1.8 [2.8] vs 0.9 [1.6]; P = .001), less daytime sleepiness (Epworth Sleepiness Scale scores, mean [SD]: 4.6 [3.3] vs 6.3 [3.7]; P < .001), more frequently reported symptoms of anxiety (34.7% vs 8.7%; P < .001), constipation (17.1% vs 8.6%; P = .028), and less frequently reported cognitive concerns when compared with unrelated healthy controls (Table 1). The LRRK2 G2019S mutation family members (mean age [SD]: 48.7 [14.1]) were younger than unrelated healthy controls (mean age [SD]: 57.1 [12.4]; P < .001).

The combination of the presence of past or current symptoms of anxiety (adjusted odds ratio [OR], 4.16; 95% confidence interval [CI], 2.01-8.63), less daytime sleepiness (Epworth Sleepiness Scale score, adjusted OR [1 unit], 0.90; 95% CI, 0.83-0.97), and higher mUPDRS score (adjusted OR [1 unit], 1.4; 95% CI, 1.20-1.67) best distinguished nonparkinsonian members of LRRK2 G2019S PD multiplex families from unrelated healthy controls. After bootstrap resampling, the model estimates remained statistically significant (Supplemental Table 1). The area under the curve of the receiver operating characteristic (ROC) curve for the model was 79.3% (see Fig. 1). In addition, the model estimates were similar if we excluded LRRK2 G2019S mutation carriers from the model (Table 3) or after frequency matching for age (Supplemental Table 2).

**Discussion**

The present study provides additional evidence that nonparkinsonian members of LRRK2 G2019S PD multiplex families share a set of clinical features independent of the G2019S mutation status. Using a large sample, we show the combined value of a set of motor and nonmotor features (presence of symptoms of anxiety, less daytime sleepiness, and a higher mUPDRS score), as opposed to individual features, to differentiate nonparkinsonian first-degree relatives of LRRK2 G2019S PD cases and unrelated healthy controls. In addition, we document that this phenotypic profile is not different in nonparkinsonian LRRK2 G2019S mutation carriers when compared with relatives who do not carry a LRRK2 mutation. Although other LRRK2 G2019S family studies report differentiating features between nonparkinsonian relatives depending on LRRK2 G2019S mutation carrier status, results have been conflicting with one study finding differences...
in motor features, whereas another study reporting only a difference in nonmotor symptoms. Contrary to these studies, we included an unrelated healthy control group. In addition, our study with a multicenter design increases the confidence that these results are not just specific to a certain region.

As a limitation to this study, we cannot exclude a volunteer bias if family members with subtle neurological symptoms were more interested in participating in the study; however, the unrelated controls may also be susceptible to this form of bias. The LRRK2 G2019S mutation family relatives were younger than unrelated healthy controls, which could be a confounder. For example, the differences found in excessive daytime sleepiness could be smaller, and the differences found in the mUPDRS could be larger. The sensitivity analyses conducted with frequency matching for age further strengthens the interpretation that the current results are likely explained by membership to a LRRK2 family, and not by age. There may also be a measurement bias for physician-rated scales such as the mUPDRS related to the knowledge of membership to a LRRK2 family in the observed nonparkinsonian group because assessments were not blinded to membership of participants in study families. The use of methods of quantitative motor assessment could provide a more sensitive and less biased approach to further assess the meaning of the observed differences in parkinsonism-related items. The absence of binding to family membership could have been associated with a greater valuation of “soft” signs observed on the neurological examination in members of LRRK2 families and with undervaluation in unrelated healthy controls. However, patient-reported outcome variables such as a history of anxiety and daytime sleepiness should be less susceptible to this type of bias.

More homogeneous phenotypic groups of nonparkinsonian relatives can be used in future studies to identify relevant gene–gene and gene–environmental interactions (epigenetics), addressing the following 2 fundamental questions: (1) To what extent are the observed set of features a manifestation of a network of genes, environmental factors, or the interaction of both that predispose to neurological dysfunction? 2) To what extent is this cluster of features ultimately associated with a higher risk of incident PD? Previous findings support the relevance of these questions. For example, the lifetime penetrance of LRRK2 mutations in relatives of multiplex PD families is greater than that reported in mutation carriers relatives of sporadic LRRK2 PD cases; this emphasizes the role of additional susceptibility factors besides the LRRK2 G2019S mutation carrier status for an increased risk of PD in multiplex families. Various studies have shown that other known PD-associated genetic factors can be associated with an increased penetrance of LRRK2 mutations and modification of age at onset of PD.

A longitudinal follow-up of the nonparkinsonian individuals considered in the present study could provide information on the risk of PD in family members presenting with the identified phenotypic profile. For example, a phenotypic profile (possibly combined with valid biomarkers in the future) is found to be associated with increased penetrance of the G2019S LRRK2 mutation, this may help to set inclusion criteria for G2019S LRRK2 mutation carriers taking part in clinical trials of future preventive therapies targeting LRRK2-associated PD. In addition, the comparison of nonparkinsonian members of LRRK2 G2019S PD multiplex families with relatives of sporadic LRRK2 G2019S PD cases, or between LRRK2 G2019S PD multiplex families with a documented high and low penetrance, may provide additional insights into how specific our findings are to families with a higher penetrance of the G2019S LRRK2 mutation. Finally, although we have studied LRRK2 G2019S multiplex families, it is possible that these results may generalize to other forms of familial PD.

References


Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s website.