Gait Alterations in Healthy Carriers of the LRRK2 G2019S Mutation

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To test for an association between the LRRK2-G2019S mutation and gait, we studied 52 first-degree relatives of patients with Parkinson’s disease (PD) who carry this mutation. An accelerometer quantified gait during usual-walking, fast-walking, and dual-tasking. Noncarriers (n = 27) and carriers (n = 25) were similar with respect to age, gender, height, and gait speed during all conditions. During dual-tasking and fast-walking, gait variability and the amplitude of the dominant peak of the accelerometer signal were significantly altered among the carriers. These findings support the possibility of previously unidentified, presymptomatic motor changes among relatives who have an increased risk of developing PD.

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The leucine-rich repeat kinase 2 (LRRK2) is an important genetic determinant of Parkinson’s disease (PD). The autosomal dominant G2019S mutation in exon 41 is associated with an increased frequency of PD in Ashkenazi Jews,1 in whom rates approach as high as 26% in familial and 14% in apparently sporadic PD. Penetrance is incomplete and age-dependent, with age-specific estimates ranging from 15% at age 50–60 years to 21% to 85% at age 70 years.2–4 The asymptomatic first-degree relatives of Ashkenazi Jewish PD patients who carry the LRRK2 G2019S mutation, of whom about 50% may carry the G2019S mutation, clearly represent a population at increased risk of developing PD.

Gait disturbances play a major role in the motor manifestation of PD. Alterations in the gait pattern frequently observed in patients with PD include decreased stride length and increased stride-to-stride variability.5–8 Changes in gait speed and variability can already be detected in recently diagnosed, de novo patients, even before any visible or symptomatic gait disturbances are reported.5,9,10

PD is known for its long prediagnostic phase.11 Efforts to identify early biomarkers and predictors of PD have identified disturbances in smell, sleep, autonomic function, and affect to help clarify the pathogenesis of the disease and inform the future development of novel therapies.11 The possibility that subtle gait alterations are also present in the prediagnostic phase of PD has never been tested. To further elucidate the role of the G2019S mutation in the development of PD, we investigated a group of first-degree relatives of Ashkenazi PD patients who are carriers of the G2019S mutation in the LRRK2 gene to test the hypothesis that challenging the central gait network with a demanding paradigm will uncover abnormalities in gait and unmask compensatory mechanisms12 among asymptomatic subjects.

Patients and Methods

Subjects

We recruited 52 healthy first-degree relatives of Ashkenazi PD patients; all of these patients were carriers of the LRRK2-G2019S mutation. The subjects were between 37 and 87 years of age and were in good health; they were free of medical complaints (eg, dementia, unstable cardiovascular disease, rheumatologic disease, and orthopedic disease), acute illness, or pain. Subjects were excluded if they had a diagnosis of PD, even early, de novo PD, a history of stroke or neurological disorders, major depression, dementia, or psychiatric diagnosis. The study was approved by both the Internal Review Board of the Tel Aviv Sourasky Medical Center and the National Israeli Committee for Human Research, and all subjects signed informed written consent.

Procedures

Subjects underwent a thorough clinical, neurological and cognitive exam including the Unified Parkinson’s Disease Rating Scale (UPDRS) motor (part III) and assessment of gait. A small, lightweight accelerometer (DynaPort; McRoberts, The Hague, Netherlands)13 was worn on the lower back during all gait measurements to quantify walking. Subjects were asked to walk along a 20-m-long, well-lit corridor for 1 minute, under the following conditions: (1) preferred, usual-walking speed; (2)

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dual-task condition, defined as performing a secondary task while walking (subtracting serial 7s); and (3) fast-speed walking.

Average stride time, gait speed, stride length, and stride time variability were evaluated. Stride time variability was determined by calculating the magnitude of the stride-to-stride fluctuations of the stride time, normalized to each subject's mean stride time (coefficient of variation $\frac{100 \times \text{standard deviation}}{\text{mean}}$). Data collected by the accelerometer was also used for spectral analysis of the calibrated acceleration signal in the locomotion band (0.5–3.0 Hz). The peak amplitude of the dominant frequency in the anterior-posterior direction was extracted from the raw signal; a sharper and narrower peak reflects a more consistent, rhythmic, and healthier gait pattern, ie, reduced gait variability and lower stride-to-stride fluctuations.

Total genomic DNA was isolated from peripheral blood leukocytes and the 6055G_A (G2019S) mutation in exon 41 of the \textit{LRRK2} gene was determined as previously described. All testing was performed in 1 session by 1 investigator who did not know the genetic status of participants until the end of the gait assessment. This information was also not disclosed to the participants.

**Statistical Analysis**

Dependent variables (eg, gait speed, stride-time variability) were checked for normality and homoscedasticity (within groups). Differences between groups (carriers of the \textit{LRRK2}-G2019S mutation vs noncarriers) were tested using repeated measures analysis of variance (group X gait condition). When differences between groups were significant ($p < 0.05$), a post hoc analysis was performed using a Student \(t\) test for further evaluation. Potential covariates (eg, age, gender, height, etc.) were evaluated and compared between groups. Scatter plots and nonparametric comparisons confirmed that any significant findings were not skewed by extreme values.. Statistical analyses were performed using SPSS version 16. To reduce the likelihood of false-positive results, given the multiple outcome measures, a Bonferroni correction was used and significance was assumed at the 0.025 level.

**Results**

Twenty-five subjects were identified as carriers of the \textit{LRRK2-G2019S} mutation. Carriers and noncarriers were similar with respect to age, gender, height, body mass index, scores on the UPDRS, and cognitive function, as well as on measures of mood, autonomic function, smell, and presence of sleep disorders (Table). Gait speed, stride time, and stride length were similar between groups under all walking conditions (see Table). In contrast, stride time variability differed across the 2 groups ($p = 0.009$). Post-hoc analysis showed that it was significantly higher (worse) during the dual-task walking condition among the carriers (1.82 ± 1.04%; $p = 0.018$). Differences were also observed during fast walking (carriers: 1.33 ± 0.58%; noncarriers: 1.05 ± 0.31%; $p = 0.03$) (Fig 1A). Spectral analysis results also revealed similar differences between the 2 groups ($p = 0.010$). The amplitude of the dominant frequency mode was smaller among the carriers, compared to noncarriers, in both the fast ($p = 0.005$) and dual-task ($p = 0.03$) conditions (see Fig 1B). Subgroup analyses revealed that even in the subset of subjects ≤60 years old (noncarriers: \(n = 21\), carriers: \(n = 20\)), in which age-associated changes were not likely to play any role, the 2 subgroups were well-matched, yet similar increases in stride-time variability were observed among the carriers during the fast ($p = 0.019$) and dual-task walking conditions ($p = 0.012$). Figure 2 shows examples of the raw accelerometer signal and the measures that were extracted to quantify gait variability in a carrier and noncarrier, illustrating the differences seen in the 2 groups.
FIGURE 2: Examples of raw signals extracted from data collected during the fast walk of (A) 1 subject in the noncarrier group and (B) 1 subject from the carrier group. Top figures demonstrate acceleration signals, the middle figures represent the change in stride time (in seconds) during the walk, and the bottom figures demonstrate the amplitude of the dominant frequency during the fast-gait condition presented as power per radians per second (PRS).

TABLE: Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Non-Carriers (n = 27)</th>
<th>Carriers (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr (range)</td>
<td>50.08 ± 8.05 (39–80)</td>
<td>53.56 ± 11.81 (37–87)</td>
<td>0.23</td>
</tr>
<tr>
<td>Gender, number (% female)</td>
<td>14 (51%)</td>
<td>11 (44%)</td>
<td>0.39</td>
</tr>
<tr>
<td>UPDRS Part III–motor</td>
<td>2.43 ± 2.72</td>
<td>3.64 ± 3.81</td>
<td>0.29</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.12 ± 11.13</td>
<td>168.31 ± 9.62</td>
<td>0.76</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.27 ± 11.81</td>
<td>79.96 ± 13.80</td>
<td>0.07a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.89 ± 3.74</td>
<td>27.36 ± 3.70</td>
<td>0.16</td>
</tr>
<tr>
<td>Cognitive function (MoCA)</td>
<td>26.34 ± 2.58</td>
<td>26.81 ± 2.42</td>
<td>0.67</td>
</tr>
<tr>
<td>Depressive symptoms (GDS)</td>
<td>4.63 ± 5.83</td>
<td>3.68 ± 2.72</td>
<td>0.46</td>
</tr>
<tr>
<td>Autonomic dysfunction (SCOPA-AUT)</td>
<td>6.18 ± 7.03</td>
<td>5.68 ± 4.20</td>
<td>0.13a</td>
</tr>
<tr>
<td>Sleep disturbances (% affected)b</td>
<td>0%</td>
<td>12%</td>
<td>0.06a</td>
</tr>
<tr>
<td>Smell (UPSIT)</td>
<td>27.85 ± 6.17</td>
<td>28.72 ± 4.12</td>
<td>0.56</td>
</tr>
<tr>
<td>Gait speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Usual walking (m/sec)</td>
<td>1.37 ± 0.19</td>
<td>1.36 ± 0.17</td>
<td>0.96</td>
</tr>
<tr>
<td>Fast walking (m/sec)</td>
<td>1.61 ± 0.21</td>
<td>1.69 ± 0.18</td>
<td>0.86</td>
</tr>
<tr>
<td>Dual task (m/sec)</td>
<td>1.21 ± 0.18</td>
<td>1.19 ± 0.16</td>
<td>0.91</td>
</tr>
<tr>
<td>Stride time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Usual walking (sec)</td>
<td>1.06 ± 0.07</td>
<td>1.05 ± 0.09</td>
<td>0.81</td>
</tr>
<tr>
<td>Fast walking (sec)</td>
<td>0.95 ± 0.06</td>
<td>0.94 ± 0.10</td>
<td>0.78</td>
</tr>
<tr>
<td>Dual task (sec)</td>
<td>1.11 ± 0.09</td>
<td>1.10 ± 0.11</td>
<td>0.94</td>
</tr>
<tr>
<td>Stride length: usual walking (cm)</td>
<td>146.42 ± 13.71</td>
<td>145.09 ± 15.60</td>
<td>0.89</td>
</tr>
</tbody>
</table>

These potential confounders, including the UPDRS motor scores, were not correlated with gait variability (p > 0.101). None of the subjects had resting tremor or had other findings that might suggest the presence of early PD.

Sleep disturbances assessed using the REM Sleep Behavior Disorder Questionnaire.

GDS = Geriatric Depression Scale; MoCA = Montreal Cognitive Assessment; SCOPA-AUT = Scales for Outcomes in Parkinson’s Disease-Autonomic Questionnaire; UPSIT = University of Pennsylvania Smell Identification Test.
Discussion
To our knowledge, this is the first report of changes in motor performance among healthy asymptomatic carriers of the LRRK2-G2019S mutation. Although results of both groups were within normative ranges, gait variability of the carriers was worse than in the noncarriers in all conditions, especially during more challenging tasks.

Stride-time variability has been shown to be a highly sensitive measure of gait consistency and stability, especially under challenging conditions. In PD, the ability to regulate stride-to-stride fluctuations decreases and gait variability increases during dual tasking as this task requires the recruitment of additional attentional resources. The poorer performance of the LRRK2-G2019S mutation carriers may be consistent, therefore with subtle abnormalities in the central gait network as manifested during the challenging conditions, thus demonstrating decreased compensatory reserve.

There are at least 2 competing explanations for the present findings. It may be possible that among the carriers, a population that is at increased risk for developing PD, the subtle changes observed in gait variability could reflect early preclinical motor alterations, ie, an early manifestation of subtle alterations to the central gait network. This intriguing possibility can only be confirmed by long-term, prospective follow-up of these subjects. Another possibility is that the gait network of the LRRK2-G2019S mutation carriers is simply different, unrelated to the future development of PD. Compensatory mechanisms may be sufficient during usual-walking, but not during more challenging conditions. The deficits observed in these first-degree relatives might therefore reflect, at least in part, an endophenotypic marker and not an early biomarker of PD.

The present observation is supported by 2 recent functional magnetic resonance imaging studies that tested upper extremity movements in asymptomatic carriers of mutations in the PARKIN or PINK1 genes. A stronger increase in movement-related activity in the right rostral cingulate motor area and left dorsal premotor cortex and increased compensatory recruitment of the rostral supplementary motor area during movements was observed compared to noncarriers. While these studies did not examine gait, the findings are consistent with the possibility of changes to the neural networks involved in motor control and perhaps in recruitment of compensatory reserves among asymptomatic mutation carriers, which may be independent of a diseased state.

The present, preliminary results should be interpreted cautiously. Still, these novel findings have implications for understanding the genotype-phenotype relation of the LRRK-G2019S mutation. The findings also support the intriguing possibility that gait dynamics during challenging conditions may serve as a new, sensitive biologic marker of presymptomatic PD. Larger scale, longitudinal studies are, however, needed to assess whether the gait changes observed are predictive of PD.

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Potential Conflicts of Interest
Nothing to report.

References
Motor Nerve Biopsy: Clinical Usefulness and Histopathological Criteria

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Early differential diagnosis of motor neuropathies (MN) and lower motor neuron diseases (LMND) is important, as prognosis and therapeutic approaches are different. We evaluated the diagnostic contribution of the biopsy of the motor branch of the obturator nerve and gracilis muscle in 21 consecutive patients in which, after proper clinical and neurophysiological studies, the differential diagnosis was still open. At baseline, motor biopsy was performed; diagnostic confirmation was obtained by 2-year clinical follow-up. Our results support the usefulness of this diagnostic procedure for selected cases of MN and LMND.

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Motor neuron disease (MND) indicates a group of neurological disorders characterized by degeneration of motor neurons. Amyotrophic lateral sclerosis (ALS) is the most common form, involving both lower motor neurons (LMN) and upper motor neurons (UMN), whose biological, psychological, and social impacts are devastating.1 ALS diagnosis is generally fairly simple,2 but may be less certain in patients presenting with sporadic progressive disease of LMN. These patients were diagnosed as “suspected ALS” according to the 1994 El-Escorial criteria but this category no longer exists in the 2000 revised criteria.3 The term lower MND (LMND) is more appropriately used to indicate this heterogeneous group of diseases, which includes progressive muscular atrophy (PMA). A substantial proportion of PMA patients develop ALS or have an “ALS-like” disease course.4 Notably, the reported percentage of misdiagnosis is 19% for PMA,5 and up to 10% for ALS (1% rediagnosed as neuropa-thy).6–8 Therefore, in some cases, only follow-up can lead to a certain diagnosis.

Motor neuropathies (MN) are an heterogeneous group of diseases primarily affecting the motor nerves. In most MN cases the absence of UMN signs and demyelinating features at nerve conduction studies lead to a straightforward diagnosis. However, demyelinating features may not always be demonstrated and purely axonal electrophysiologic findings are found in selected cases, some responding to intravenous immunoglobulin therapy.9–11

Early differential diagnosis between LMND and MN is important, as prognosis and therapeutic approach are different; moreover, current and future therapies might be more effective in the first stages of disease.12

The morphological aspects of the motor branch of the obturator nerve have been shown to differ in patients with a definite diagnosis of MN or MND; however, the


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