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Multimodal oculomotor assessment reveals prodromal markers of Parkinson's disease in non-manifesting *LRRK2* G2019S mutation carriers

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Oculomotor behaviour changes in patients with Parkinson's disease (PD) are a promising source of prodromal disease markers. Capitalizing on this phenomenon to facilitate early diagnosis requires oculomotor assessment in prodromal cohorts. We examined oculomotor behaviour in non-manifesting *LRRK2* G2019S mutation carriers (LRRK2-NM), who have heightened PD risk. Seventeen LRRK2-NM participants, 47 patients with idiopathic PD, and 63 healthy age-matched control participants completed an interleaved pro- and antisaccade task while undergoing video-based eye-tracking. We analyzed between-group differences in saccade, pupil, blink, and fixation acquisition behaviour. Patients with PD showed previously demonstrated abnormalities (saccade hypometria, antisaccade errors). Relative to controls, LRRK2-NM participants and patients with PD both displayed increased short-latency prosaccades and reduced pupil velocity, plus altered fixation acquisition—less preemptive returning of gaze to the future fixation point location. Interestingly, the effect on blink probability was opposite—higher than controls in LRRK2-NM participants but lower in patients with PD. Future longitudinal studies must confirm the viability of these features as prodromal PD markers.

Parkinson's disease (PD) is clinically diagnosed based on history and physical examination findings, including the presence of motor symptoms and signs such as bradykinesia, resting tremor, and/or rigidity[1,2]. Although these signs typically manifest once neuropathology reaches nigrostriatal circuitry[3], earlier damage to other regions can produce other signs and symptoms and the development of a prodromal state[4]. Earlier identification of prodromal alterations would support faster diagnosis, earlier treatment initiation, and development of disease-modifying therapies, as illumination of prodromal disease processes could enhance pathological targeting and enable trials of prodromal treatments. However, the difficulty of predicting who will develop idiopathic PD impedes clinical characterization of its prodromal phase. Studying populations with elevated PD risk mitigates this issue and facilitates exploration of prodromal behavioural markers.

The G2019S mutation in the *LRRK2* gene is associated with autosomal dominantly inherited PD, although penetrance is incomplete, and is found in 1% of sporadic and 4% of hereditary PD cases[5,6]. Phenotypically, LRRK2-associated PD overlaps significantly with the idiopathic form, with minor differences in presentation and symptom severity[5,7]. Although prodromal *LRRK2*-associated PD is difficult to assess due to infrequency and incomplete penetrance, its motor abnormalities likely overlap with prodromal idiopathic PD, although nonmotor features such as hyposmia and REM sleep behaviour disorder (RBD) may be rarer[7,8]. Because idiopathic PD is

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nearly impossible to predict at very early stages, these factors make non-manifesting *LRRK2* G2019S carriers (LRRK2-NM) an ideal population to investigate prodromal PD. Examining LRRK2-NM individuals prior to the onset of clinically manifest parkinsonism will illuminate how pathological processes begin modifying neural function pre-diagnosis.

Oculomotor assessment has been widely used to study neurodegenerative disease because circuitry controlling saccades, pupil responses, and eye blinks intersects considerably with brain regions disrupted by pathology[9]. PD has been particularly frequently studied using prosaccade and antisaccade task paradigms[10,11]. Briefly, prosaccades require participants to look at a peripheral stimulus when it appears, while antisaccades require them to look in the opposite direction. They assess the integrity of visuomotor and cognitive processes respectively[12] and can also expose irregularities in pupil responses[13] and eye blinks[14] that reveal disease-related modulation of neural circuits. PD typically increases antisaccade error rates, especially in cognitively impaired patients[11] and slows correct antisaccade reaction times[10,15–17]. Studies find both increased rates of short-latency prosaccades and prolonged prosaccade reaction time; this may be partially related to medication given findings that dopaminergic drugs slow prosaccade reaction time[18,19] and could indicate a speeding effect of the disease itself on visually-driven saccades. Broadly, these results denote reduced inhibitory control and upregulation of visual input in driving saccades; such upregulation could be an early compensatory mechanism for disease-driven slowing[20,21]. Saccade hypometria is also common in patients with PD[16,18,22]. Pupil dilation responses linked to voluntary control are blunted in patients with PD[23] and blink rate is generally diminished[24,25]. Finally, saccadic intrusions may be more frequent in patients with PD, reducing fixation stability[26,27], although this is less pronounced in early disease and overlaps considerably with intrusion frequency in healthy controls[28].

Although much rarer, a few studies have examined oculomotor behaviour in non-manifesting carriers of PD risk mutations. One recent study specifically evaluated non-manifesting *LRRK2* G2019S mutation carriers[29], who demonstrated fixation instability, increased prosaccade latency, and vertical saccade hypometria. However, very few trials were performed, and pupil and blink responses were not assessed. Saccade alterations in other asymptomatic carriers of PD risk mutations are mixed, with *Parkin* mutation carriers displaying none[30,31], while *PINK1* mutation carriers had elevated prosaccade latency and disrupted gaze shifts between trials[32].

The current study describes a novel analysis: fixation acquisition. This behaviour occurs when participants return gaze to the screen centre after one trial in preparation for the next. Although saccade behaviour is not typically assessed during this epoch, unprocessed data from our lab suggested it contains structured behaviour that changes in patients (see Methods and Supplementary Background). Because LRRK2-NM individuals have minimal pathology[8,33] and might therefore display more understated or different behavioural alterations than patients with PD, we analyzed fixation acquisition to comprehensively evaluate saccade function for subtle alterations.

Here we describe saccade, pupil, blink, and fixation acquisition behaviour in LRRK2-NM participants and compare it to healthy age- and sex-matched controls and patients with sporadic idiopathic PD. This work expands significantly on the single existing study of oculomotor behaviour in LRRK2-NM individuals by evaluating pupil responses, blinks, fixation acquisition, and six times more prosaccade and antisaccade trials. We selected prosaccades, antisaccades, pupil responses, and blinks for analysis due to their established alteration in patients with PD relative to controls. Any behavioural similarities to patients with PD, and differences from controls, represent potential markers of early PD pathology. Therefore, we expect LRRK2-NM individuals to demonstrate similar but smaller alterations relative to patients with manifest PD (e.g. saccade hypometria, blunted pupil dilation, reduced blink rate), especially those associated with early disease processes or compensatory mechanisms (e.g. prosaccade speeding) but not late disease (e.g. antisaccade errors). Due to its novelty, the neural underpinnings and pathological modification of fixation acquisition are unclear; however, unpublished observations from identical fixation acquisition analysis performed using data from our lab reveal slower, less frequent predictive fixation in patients with PD, which we therefore expect to be intermediate in LRRK2-NM.

Results

Participant characteristics

Cohort demographics are shown in Table 1 and Supplementary Fig. 1. The groups differed significantly in age ($F(2, 124) = 5.05$, $p = 0.008$, $\eta^2 = 0.08$) and Montreal Cognitive Assessment (MoCA)[34] scores ($H(2) = 10.28$, $p = 0.006$, $\eta^2 = 0.07$) with the PD group significantly older than controls ($p = 0.007$) and having significantly lower MoCA scores ($p = 0.005$). There were no other significant between-group differences. The groups also differed in sex ($\chi^2(2) = 13.02$, $p = 0.001$, Cramer's V = 0.22) due to the male-dominated PD group, but oculomotor measures are largely unaffected by sex, especially in older adults[14,35]. Although idiopathic PD is more frequent in males, *LRRK2*-associated PD is equally common between sexes[5,36]. Therefore, the higher proportion of female participants in the LRRK2-NM cohort does not reduce their collective probability of PD manifestation or diminish their suitability as a prodromal cohort. Patients with PD had a mean Movement Disorder Society-United Parkinson's Disease Rating Scale (MDS-UPDRS) Part III score of 19.3, indicating mild severity.

LRRK2-NM participants had a mean prodromal score of 22.6 (SD = 32.2; range = 0.2–101.4); scores were available for 10 of 17 participants. No participants met age-based prodromal score cutoffs for 80% probability of prodromal PD[37], with the closest participant having a probability of 67%. Therefore, the LRRK2-NM participants were generally unlikely to have prodromal PD, and the subset who will eventually convert are at preclinical stages or earlier (i.e. no evident signs or symptoms, although neurodegeneration may have begun)[37]. This is perhaps unsurprising considering the limited prevalence (~28%) of *LRRK2*-associated PD at the mean age of this cohort[5]. Prodromal scores did not significantly correlate with oculomotor behaviour (Supplementary Table 5).

Saccade analysis

We hypothesized that LRRK2-NM participants would display analogous but less severe saccade alterations relative to patients with PD, especially those most linked to early disease (e.g. reduced prosaccade reaction time). On correct prosaccade trials (Fig. 1A), the LRRK2-NM curve was significantly higher than the control curve from saccadic reaction time (SRT) −50–124ms ($p = 0.020$, cluster sum = −259.07), which would include both anticipatory and express-latency saccades. Similarly, the PD curve was significantly higher than the control curve from SRT 10-158 ms ($p = 0.023$,

Table 1 | Demographic characteristics of study cohort

Group	Number of participants (number, % of female participants)	Age in years (mean ± SD)	MoCA score (mean ± SD)	Prodromal score (mean ± SD)	MDS-UPDRS Part III score (mean ± SD)
Controls	63 (35 F, 55.6%)	57.6 ± 13.0	28.3 ± 1.4	–	–
LRRK2-NM	17 (10 F, 58.8%)	58.4 ± 13.4	26.7 ± 3.2	22.6 ± 32.2	–
PD	47 (11 F, 23.4%)	64.2 ± 6.7	26.8 ± 2.5	–	19.3 ± 8.3

LRRK2-NM: non-manifesting LRRK2 mutation carriers; PD: patients with Parkinson's disease; SD: standard deviation; MoCA: Montreal Cognitive Assessment. Note that MoCA scores and prodromal scores were unavailable for 6 and 7 LRRK2-NM participants respectively. MDS-UPDRS Part III score was unavailable for 1 patient with PD.

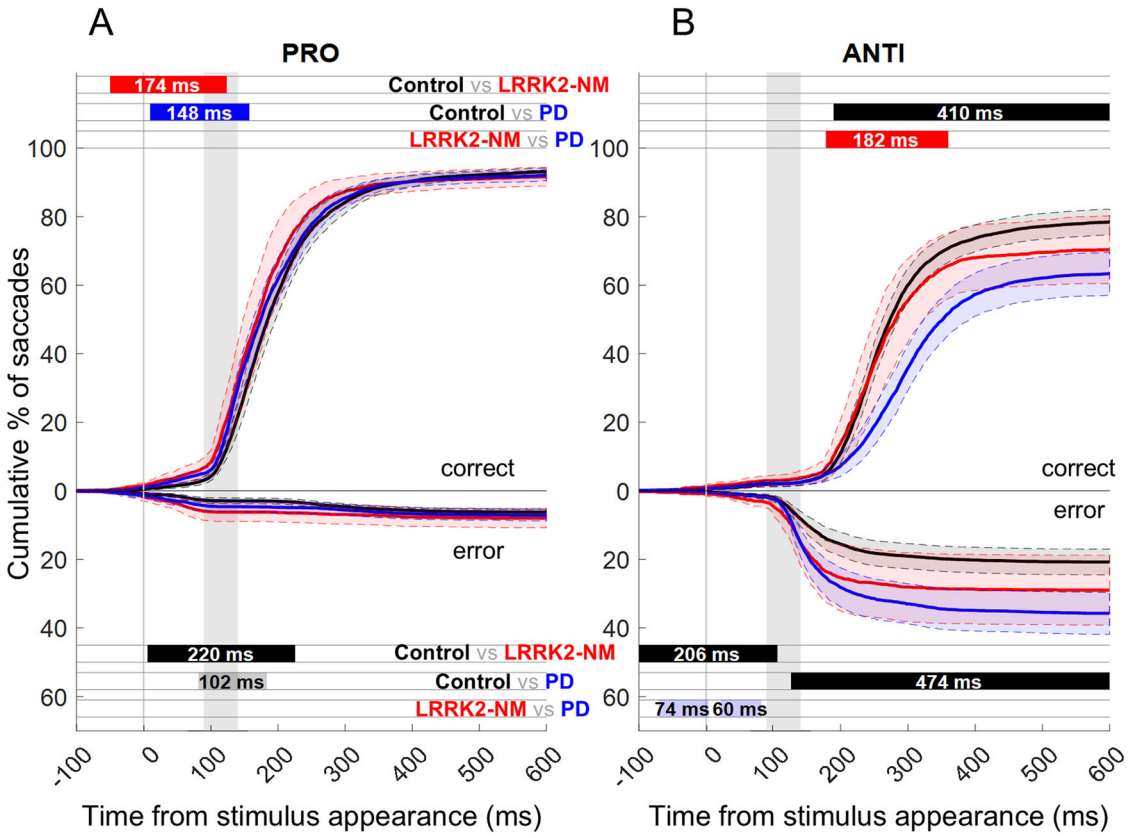

Fig. 1 | Cumulative distributions of saccade reaction time and between-group differences. Cumulative distributions of correct and incorrect saccade reaction time and periods of significant between-group difference in (**A**) prosaccade trials and (**B**) antisaccade trials. Shaded regions bounded by dashed lines indicate 95% confidence intervals. Data above 0 on the ordinate represent correct responses; data below represent errors. Vertical grey line indicates stimulus appearance at 0 ms; shaded grey box indicates express saccade epoch (90–140 ms). Sets of three horizontal tracks above and below data indicate between-group comparisons (in each set, top: controls vs LRRK2-NM participants; middle: controls vs patients with PD; bottom: LRRK2-NM participants vs patients with PD). All significant periods of difference ($p \leq 0.025$) are shown by dark filled bars with colour corresponding to the group whose curve is higher during that time. Trending periods ($p < 0.05$) are indicated by lighter bars. PRO prosaccade; ANTI antisaccade; LRRK2-NM LRRK2 non-manifesting carriers; PD Parkinson's disease.

cluster sum = −208.19). For error trials, the control curve was significantly higher than the LRRK2-NM curve from SRT 6 ms to 226 ms ($p = 0.015$, cluster sum = 259.09), denoting fewer errors in controls, which encompassed some anticipatory, express, and fast regular-latency saccades. A cluster from SRT 82-184 ms showing the control curve higher than the PD curve also trended towards but did not reach statistical significance ($p = 0.034$, cluster sum=105.67). These results suggest that LRRK2-NM participants made more prosaccades, both correct and errors, than controls at short latencies, and that this tendency was also present in patients with PD for correct trials.

Correct prosaccades differed in amplitude between groups ($F(2, 124) = 12.40$, $p < 0.001$, $\eta^2 = 0.17$). In line with many previous findings[11], patients with PD (median 8.9°, IQR 1.1°, range 6.1–11.9°) made significantly smaller prosaccades than controls (median 9.5°, IQR 0.7°, range 8.1–11.1°) to a target presented at 10° eccentricity ($p < 0.001$) (Supplementary Table 2, Supplementary Fig. 3). The median amplitude for LRRK2-NM participants (median 9.2°, IQR 1.1°, range 8.1–10.5°) was not significantly different from controls (median 9.5°, IQR 0.7°, range 8.1–11.1°) ($p = 0.40$).

On correct antisaccade trials (Fig. 1B), there were no significant differences between LRRK2-NM participants and controls; however, the control curve was significantly higher than the PD curve from SRT 190–600 ms ($p < 0.001$, cluster sum = 875.53), as was the LRRK2-NM curve from SRT 178–360 ms ($p = 0.024$, cluster sum = 238.83), indicating more errors and slower correct antisaccades in patients with PD. For antisaccade error trials, the control curve was significantly higher than the LRRK2-NM curve from SRT −100-106ms ($p = 0.017$, cluster sum=293.86), indicating more errors in LRRK2-NM participants and encompassing mostly anticipatory saccades. Although not statistically significant, two short clusters of similar SRT showed the PD curve higher than the LRRK2-NM curve (SRT −70-4ms, $p = 0.033$, cluster sum = −105.25; and SRT 22–82 ms; $p = 0.041$, cluster sum = −71.88). Like correct antisaccade trials, the control curve was also significantly higher than the PD curve between SRT 126-600 ms ($p < 0.001$, cluster sum = 919.31), indicating more errors in patients with PD. Together, these results indicate that LRRK2-NM participants made more short-latency antisaccade errors than controls and that patients with PD made more errors than controls at longer latencies.

Pupil analysis

Given the previously observed blunting of pupil dilation responses in patients with PD[23], we expected similar but less severe dampening in LRRK2-NM participants. We observed blunting in the pupil responses of patients with PD (Fig. 2A, B). The PD pupil size curve was significantly higher than the control curve from 492-828 ms after fixation point (FP) appearance on antisaccade trials (Fig. 2B) ($p = 0.025$, cluster sum = −391.34). A similar epoch neared but did not reach statistical significance on prosaccade trials (Fig. 2A) ($p = 0.036$, cluster sum = −206.23). There was also a non-significant period during the dilation phase on antisaccade trials from 1016-1222 ms after FP appearance during which the LRRK2-NM curve was higher than the control curve ($p = 0.036$, cluster sum = −237.73).

On prosaccade trials and antisaccade trials, pupil velocity for patients with PD was significantly slower than controls during constriction (Fig. 2C, D) (prosaccade: 422-630 ms after FP appearance, $p = 0.017$, cluster sum = −249.70; antisaccade: 394–640 ms after FP appearance, $p = 0.017$,

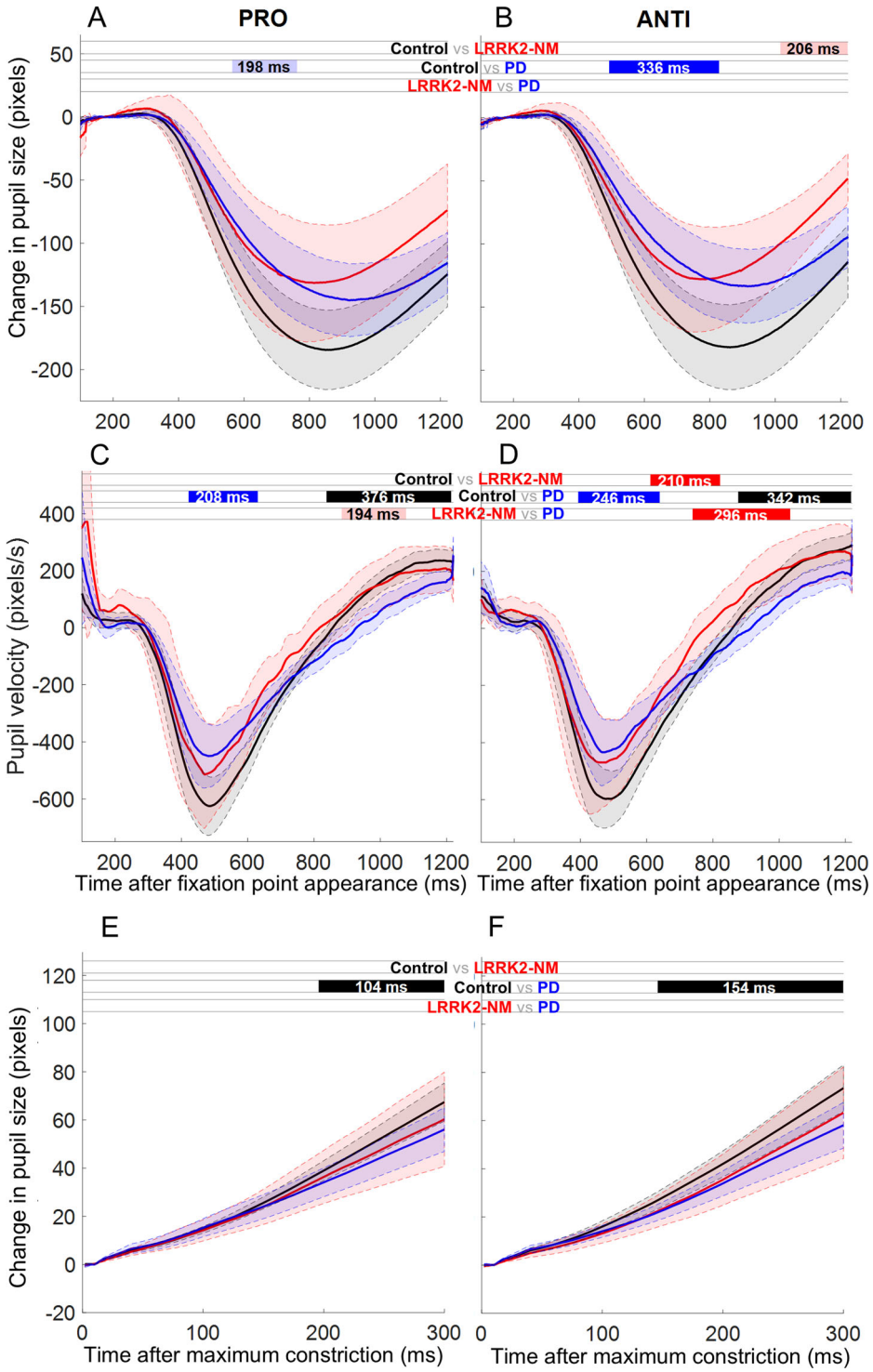

Fig. 2 | Pupil size, velocity, and dilation responses and between-group differences. Pupil analysis in prosaccade trials (left column) and antisaccade trials (right column), including (**A**) and (**B**) pupil size; (**C**) and (**D**) pupil velocity; (**E**) and (**F**) pupil dilation response only. Shaded regions bounded by dashed lines indicate 95% confidence intervals. Horizontal tracks above data display between-group comparisons (top: controls vs LRRK2-NM participants; middle: controls vs patients with PD; bottom: LRRK2-NM participants vs patients with PD). All significant periods of difference ($p \leq 0.025$) are shown by dark filled bars with colour corresponding to the group whose curve is higher during that time. Trending periods ($p < 0.05$) are indicated by lighter bars. PRO prosaccade; ANTI antisaccade; LRRK2-NM LRRK2 non-manifesting carriers; PD Parkinson's disease.

cluster sum = −306.42) (note that although the PD curve is higher at these times, this indicates slower velocity, as velocity is negative during constriction). Similarly, patients with PD had significantly slower velocity than controls during dilation on both trial types (prosaccade: 838–1214 ms after FP appearance, $p < 0.001$, cluster sum = 641.41; antisaccade: 876-1218 ms after FP appearance, $p = 0.001$, cluster sum = 486.15). Patients with PD also tended to be slower than LRRK2-NM participants during dilation, with a significant cluster occurring on antisaccade trials (738–1034 ms after FP appearance, $p = 0.008$, cluster sum = 411.18) and a similar but non-significant cluster on prosaccade trials (884–1078 ms after FP appearance, $p = 0.035$, cluster sum = 226.06). Finally, the LRRK2-NM curve was significantly higher than the control curve on antisaccade trials from 612 to 822 ms after FP appearance ($p = 0.025$, cluster sum = –228.49), which indicates slower velocity in LRRK2-NM participants surrounding the period of maximum constriction (as velocity values were primarily negative during this epoch).

Isolating the dilation phase alone (Fig. 2E, F) showed that patients with PD had significantly less dilation than controls on both prosaccade and antisaccade trials (prosaccade: 196–300 ms after maximum constriction, $p = 0.025$, cluster sum = 109.60; antisaccade: 146-300 ms after maximum constriction, $p = 0.021$, cluster sum = 353.96). There were no differences between LRRK2-NM and control participants.

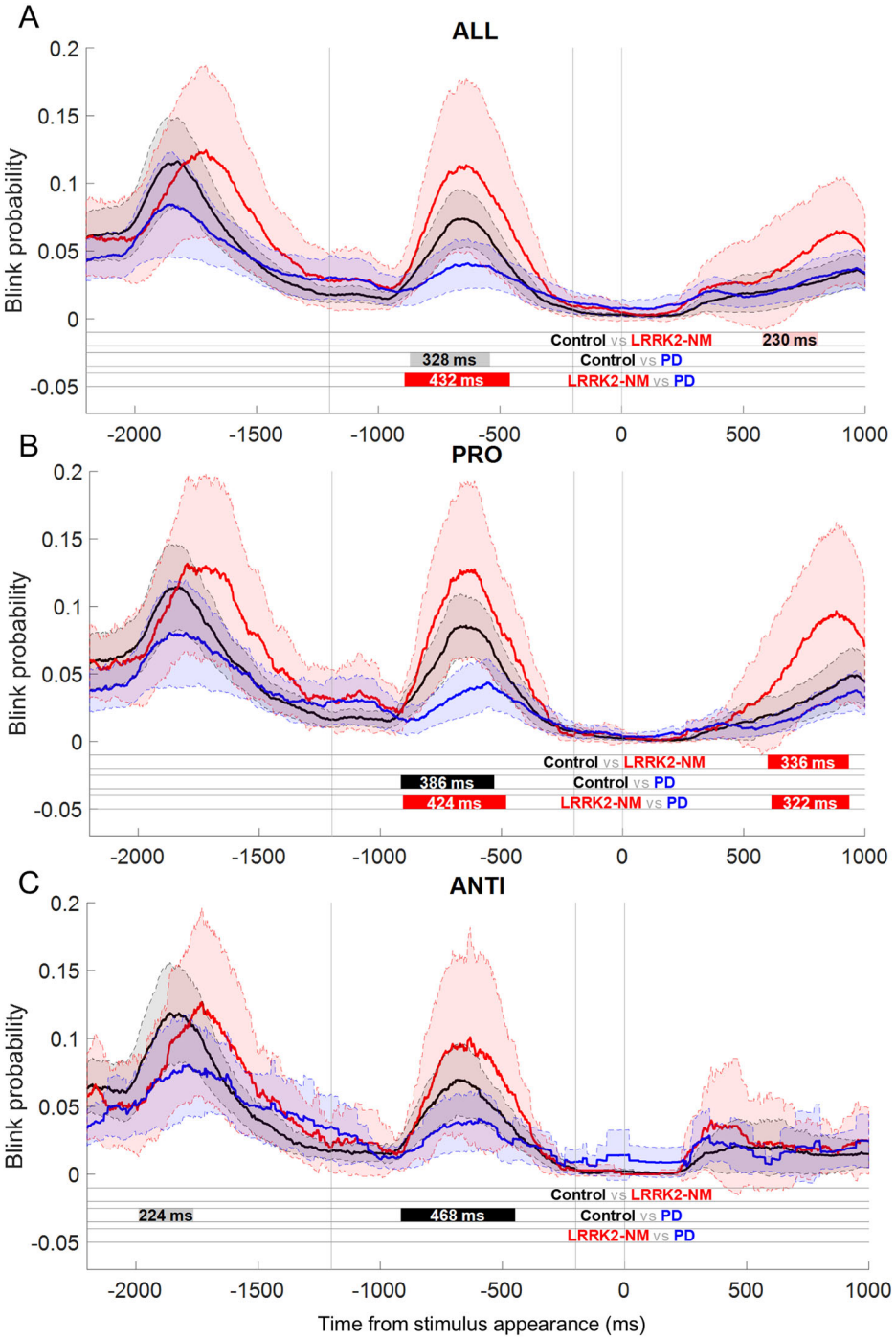

Fig. 3 | Blink probability and between-group differences. Blink probability curves in (**A**) all trials combined; (**B**) correct prosaccade trials only; (**C**) correct antisaccade trials only. Shaded regions bounded by dashed lines indicate 95% confidence intervals. Grey vertical lines indicate, from left to right: fixation point appearance (−1200 ms), fixation point disappearance (−200 ms), and stimulus appearance (0 ms). Horizontal tracks below data indicate between-group comparisons (top: controls vs LRRK2-NM participants; middle: controls vs patients with PD; bottom: LRRK2-NM participants vs patients with PD). All significant periods of difference ($p \leq 0.025$) are shown by dark filled bars with colour corresponding to the group whose curve is higher during that time. Trending periods ($p < 0.05$) are indicated by lighter bars. PRO prosaccade; ANTI antisaccade; LRRK2-NM LRRK2 non-manifesting carriers; PD Parkinson's disease.

Blink analysis

We hypothesized that blink probability would be reduced in LRRK2-NM participants, but less than in patients with PD. However, our results proved contrary to this hypothesis. When prosaccade and antisaccade trials were pooled (Fig. 3A), the only significant difference was that LRRK2-NM participants had significantly higher blink probability than patients with PD during the fixation period, between 892–460 ms prior to stimulus appearance ($p = 0.013$, cluster sum = 587.58). A similar but non-significant cluster suggested higher blink probability in controls relative to patients with PD from 870 to 542 ms before stimulus appearance ($p = 0.035$, cluster sum = 416.52). An additional non-significant cluster suggested higher blink probability in LRRK2-NM participants than controls during stimulus presentation, from 578-808 ms after stimulus appearance ($p = 0.048$, cluster sum = −290.84).

When prosaccade trials were isolated (Fig. 3B), these results became more pronounced. LRRK2-NM participants had significantly higher blink probability than controls from 598 to 934 ms after stimulus appearance ($p = 0.020$, cluster sum = −434.26) and significantly higher than patients with PD during a similar period, 614–36 ms after stimulus appearance ($p = 0.012$, cluster sum = 403.32). During the fixation period, patients with PD had significantly lower blink probability than controls (916–530 ms before stimulus appearance, $p = 0.005$, cluster sum = 546.34) and LRRK2-NM participants (906–482 ms before stimulus appearance, $p = 0.003$, cluster sum=655.81).

For antisaccade trials (Fig. 3C), the only significant result was that patients with PD had significantly lower blink probability than controls during the fixation period, 916-448 ms before stimulus appearance ($p = 0.006$, cluster sum = 623.87). A non-significant cluster suggested

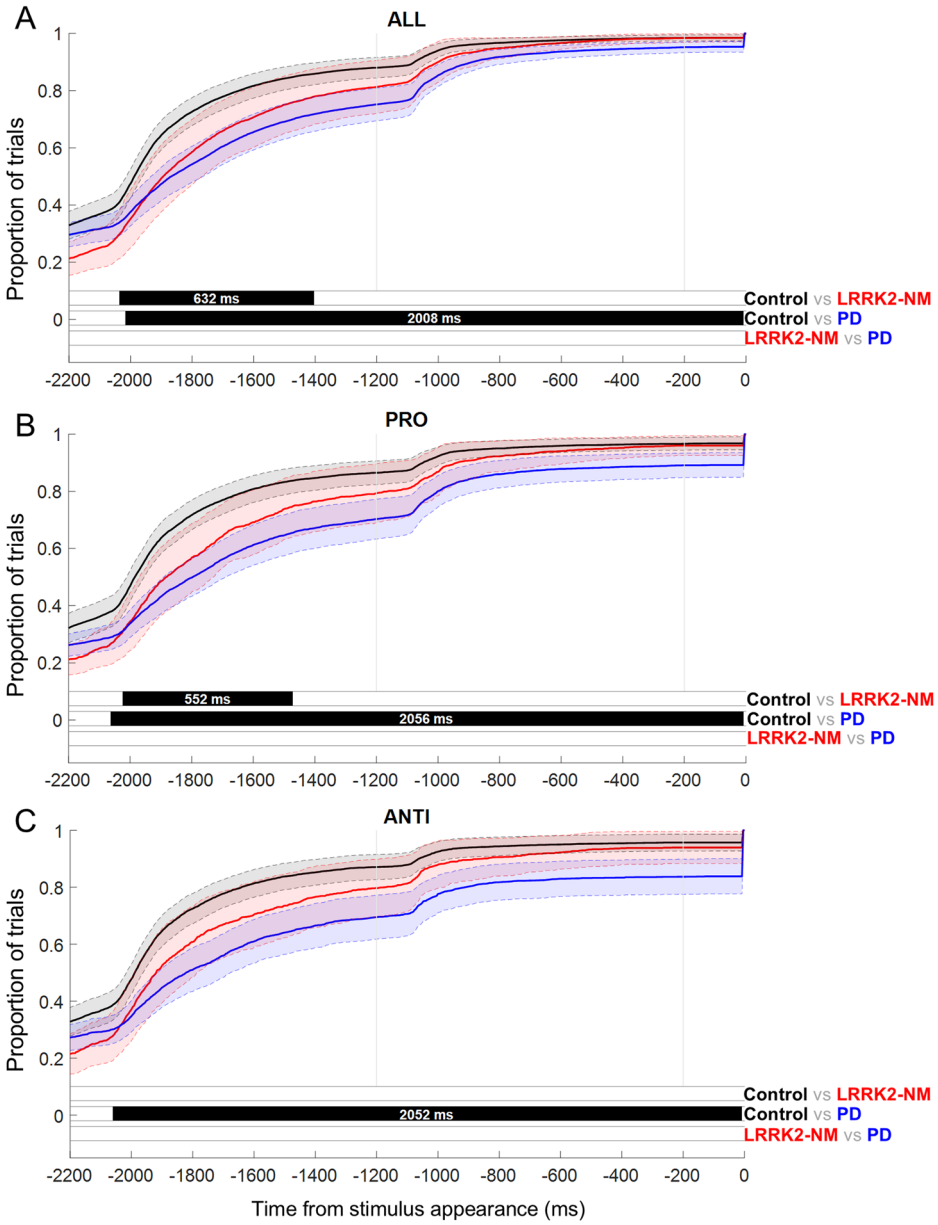

Fig. 4 | Fixation acquisition and between-group differences. Fixation acquisition curves in (**A**) all trials combined; (**B**) correct prosaccade trials only; (**C**) correct antisaccade trials only. Vertical grey lines, from left to right, indicate fixation point appearance (−1200 ms) and disappearance (−200 ms). Shaded regions bounded by dashed lines indicate 95% confidence intervals. Horizontal tracks below data indicate between-group comparisons (top: controls vs LRRK2-NM participants; middle: controls vs patients with PD; bottom: LRRK2-NM participants vs patients with PD). All significant periods of difference ($p \leq 0.025$) are shown by dark filled bars with colour corresponding to the group whose curve is higher during that time. Trending periods ($p < 0.05$) are indicated by lighter bars. PRO prosaccade; ANTI antisaccade; LRRK2-NM LRRK2 non-manifesting carriers; PD Parkinson's disease.

patients with PD may have lower blink probability than controls during the intertrial interval (ITI), 1988-1764ms before stimulus appearance ($p = 0.040$, cluster sum = 279.28).

Fixation acquisition analysis

We hypothesized that patients with PD would make fewer predictive saccades to centre than controls and that LRRK2-NM participants would fall between them. When both prosaccade and antisaccade trials were combined (Fig. 4A), the control curve was significantly higher than both the LRRK2-NM curve during the ITI (between 2036–1404 ms prior to stimulus appearance; $p = 0.023$, cluster sum = 353.95) and the PD curve throughout the ITI and fixation period (between 2016-8 ms prior to stimulus appearance; $p < 0.001$, cluster sum=2040.90), indicating that LRRK2-NM participants and patients with PD less frequently predicted FP appearance than controls, and patients with PD also reactively looked at the FP less often once it appeared. These results did not change when prosaccade trials were isolated (Fig. 4B), with the control curve significantly higher than the LRRK2-NM curve from 2024–1472 ms before stimulus appearance ($p = 0.024$, cluster sum = 306.27) and significantly higher than the PD curve from 2064–8 ms before stimulus appearance ($p < 0.001$, cluster sum = 2106.30). For antisaccade trials alone, the control curve was significantly higher than the PD curve from 2060-8 ms before stimulus appearance ($p < 0.001$, cluster sum = 2122.00), but there were no differences between LRRK2-NM participants and controls. No significant differences between LRRK2-NM participants and patients with PD were observed across any trial type.

Discussion

Here, we describe behavioural differences between LRRK2-NM individuals and age-matched control participants that could denote prodromal PD, especially when accompanied by similar differences in patients with manifest PD. Firstly, we observed increased short-latency correct and incorrect prosaccades and increased short-latency antisaccade errors in LRRK2-NM participants and increased short-latency correct prosaccades in patients with PD (Fig. 1A, B), as we hypothesized due to prosaccade-specific speeding in early and/or unmedicated PD. Additionally, both LRRK2-NM participants and patients with PD demonstrated pupil velocity abnormalities. Patients with PD had slower velocity than controls during the constriction and dilation phases across both prosaccade (Fig. 2C) and

antisaccade (Fig. 2D) trials, while LRRK2-NM participants had slower velocity during maximum constriction for antisaccades only. Pupil size did not differ between LRRK2-NM participants and controls, although patients with PD had smaller pupils during both constriction and dilation. This result corroborates previous findings of pupil response blunting in patients with PD[23] and our hypothesis that LRRK2-NM participants might show similar blunting, although we observed only velocity blunting occurring during a different window than in patients with PD. Unexpectedly, we observed higher blink probability in LRRK2-NM participants than controls during stimulus presentation on prosaccade trials (Fig. 3B). This opposed our hypothesis that they would blink less, as patients with PD did during the fixation period (Fig. 3). Finally, we also saw abnormal fixation acquisition behaviour: less prediction of FP appearance, as hypothesized (Fig. 4). Importantly, all analyses showed differences between controls and the two PD-related groups, which were similar although not identical to one another.

Very few studies of oculomotor behaviour in asymptomatic genetic PD risk cohorts exist. A single directly comparable study[29] found that LRRK2-NM individuals demonstrated fixation instability (increased microsaccade amplitude and velocity, more square-wave jerks), vertical saccade hypometria, and increased prosaccade and memory-guided saccade latency. Microsaccades, saccades, and saccadic intrusions such as square-wave jerks share generative neural mechanisms, and microsaccades have been associated with attentional processes[38]. Although we did not assess sustained fixation, the potential relationship between fixation acquisition and attentional control (see "Fixation acquisition" below) could align our results with the previously reported fixation instability. We did not examine vertical saccades and did not find a significant difference in horizontal saccade amplitude between controls and LRRK2-NM participants. We also did not observe increased prosaccade latency; opposingly, we observed elevated rates of short-latency prosaccades. Notably, the previous study comprised many fewer prosaccade trials (12 horizontal, 8 vertical) than our study (120 horizontal), which might introduce considerable variability and reduce the reliability of the conclusions regarding prosaccade hypometria and latency. One additional study of 21 healthy relatives of patients with PD included two LRRK2-NM participants and found that relatives made more sequences of multiple step saccades than unrelated control participants, plus more antisaccade errors, with no effects on prosaccade latency or gain[39]. However, these results are difficult to generalize due to the nonspecific nature of the cohort. Studies of people with *PINK1* and *Parkin* mutations are less comparable, but provide some insight into potential shared mechanisms of prodromal disease; see Supplementary Discussion. Overall, given the comparatively few parameters and effectors analyzed in these studies, the current work represents a significant expansion of existing literature regarding oculomotor behaviour in LRRK2-NM individuals specifically and prodromal monogenetic PD broadly.

Patients with idiopathic PD typically have increased antisaccade error rates and slower correct antisaccade reaction time[10], which we replicated (Fig. 1B). Some studies find elevated short-latency saccades in patients with PD[16,17,20,22,40], which we observed for correct prosaccade trials. Additionally, we replicate consistent observations of saccade hypometria[10,16,18,22,41]. Pupil constriction and dilation during pro- and antisaccade tasks are blunted in patients with PD, and pupillary light reflex velocity reduced[23,42]. We observed reduced pupil velocity and dilation size in patients with PD but reduced velocity during antisaccades alone for LRRK2-NM individuals. The constriction component of the pupil response during pro- and antisaccades reflects the pupillary light reflex and is modulated by stimulus salience, whereas the dilation component reflects motor preparation[13]. Therefore, our results indicate disrupted pupillary light reflex and impaired motor preparation in patients with PD. Antisaccade trials increase preparatory requirements relative to prosaccade trials[43,44], which may be dampened in patients with PD[17]. The effect in LRRK2-NM individuals might suggest subtle preparatory deficits observable in tasks with elevated preparatory requirements but too weak to modulate pupil size. Spontaneous blink rate is typically reduced in patients with PD[22,24,25] although some display elevated rates[24,45]. An identical task generated reduced ITI blink rates in patients with PD but did not assess the fixation epoch[22]; we observed reduced blink probability in patients with PD during fixation only. Dopaminergic treatment normalizes blink rate[24], which could partially account for our finding that ITI blink probability did not differ given that many patients in the previous study were unmedicated. The blink suppression we observed might indicate that patients with PD compensate for their reduced preparatory abilities by maximizing visual input; alternatively, since blink rate shows weak modulation by sex during fixation[46], inter-cohort sex differences may drive blink probability differences. However, in a large-scale study of healthy controls performing an identical IPAST task, sex differences were minimal in older adults[14].

Our novel analysis of fixation acquisition revealed another potential prodromal behavioural marker. LRRK2-NM individuals, like patients with PD, predictively re-centred their gaze less than controls (Fig. 4). The fixation period is usually ignored by studies of pro- and antisaccades, except in analysis of pathophysiological fixation instability (e.g. square-wave jerk) or FP disappearance effects. Patients with PD successfully performed prosaccades, so their reduced prediction of the FP is probably linked to attentional or cognitive deficit rather than visual or motor. Gaze shifts away from fixation during pro- and antisaccades are more frequent in cognitively impaired participants[11] and those with ADHD[47], suggesting a relationship to higher-order cortical processing. However, the exact nature of this relationship is unclear and could be mediated by various cognitive processes such as attentional flexibility or goal-oriented task preparation. The reduction in predictive fixation acquisition observed in LRRK2-NM participants, especially coupled with increased post-stimulus blinks, might suggest attentional disengagement between trials. Alternatively, the highly temporally predictable task may not induce rhythmic patterns of behaviour in LRRK2-NM individuals as it does in controls. More investigation of the factors modulating fixation acquisition behaviour is required.

LRRK2-associated PD generally resembles idiopathic PD in clinical presentation and treatment response[5,7,8]; due to these similarities, LRRK2-NM individuals are important to the characterization of prodromal PD. Most PD cases with the *LRRK2* G2019S mutation exhibit typical α-synuclein-positive Lewy pathology (Lewy bodies (LBs) and Lewy neurites), although some cases of bland nigral degeneration or pure tau pathology have been observed; cardinal motor symptoms typically occur regardless of Lewy pathology, but nonmotor symptoms are less frequent in its absence[48], in line with findings that cortical LB load correlates with cognitive impairment in idiopathic PD[49]. Although encompassing few patients, neuropathological studies find LBs are common in the brainstem and are sometimes more diffuse, additionally involving limbic and cortical regions[50,51], suggesting that disease spreads from brainstem to cortex. Therefore, brainstem-related abnormalities might be among the first signs of disease in presymptomatic G2019S carriers, with the possible addition of deficits related to other subcortical structures such as basal ganglia. Consistent with this, we noted slowed pupil velocity in both patients with PD and LRRK2-NM participants, with LRRK2-NM participants slowing specifically at the beginning of the dilation during antisaccade trials. Pupil size is proximally controlled by brainstem circuitry, with activity in the locus coeruleus (LC) particularly related to dilation[52,53]. *LRRK2*-mediated brainstem LB pathology might therefore produce slowed dilation velocity by limiting LC activity. LC displays tonic activity correlated to baseline pupil diameter, which was unaltered in patients with PD (Supplementary Table 3), but phasic LC activity correlates to task-related pupil dilation and may be specifically affected by PD[54,55].

We observed elevated short-latency prosaccades in LRRK2-NM participants, which suggests increased impulsivity (more guesses) and/or enhanced visuomotor processing driving express-latency saccades to visual stimuli. The neural basis of prosaccade speeding in patients with PD is unclear but could be a compensation for disease-induced bradykinesia[20]. Normal error rates in LRRK2-NM participants suggest disease processes are not severely affecting frontostriatal circuits, whose integrity is important in inhibiting prepotent saccade responses and generating correct

antisaccades[12,56]. In accordance, greater neuronal loss in LC relative to substantia nigra has been observed in patients with idiopathic PD, suggesting earlier LC involvement[57] which would produce pupil abnormalities before antisaccade deficits. Similarly, reduced blinking in patients with PD likely relates to dopaminergic neuronal loss since it is normalized by dopaminergic treatment[24]. Therefore, largely normal blink behaviour in LRRK2-NM individuals harmonizes with limited nigrostriatal pathology. Interestingly, however, LRRK2-NM participants blinked more than controls following successful prosaccades to the stimulus. Spontaneous blinking has been linked to the allocation of attentional resources via frontostriatal dopaminergic pathways[14,58]; a compensatory mechanism for mild nigrostriatal pathology could broadly upregulate dopaminergic transmission such that task responses remain successful but blinks are elevated. As fixation acquisition is a novel measure, discussion of its underlying pathology is inherently speculative; see Supplementary Discussion.

The biggest limitation of our study is the small size of the LRRK2-NM cohort, due to the rarity of the *LRRK2* G2019S mutation and associated recruitment challenges. Relatedly, since *LRRK2* G2019S has incomplete penetrance, the actual proportion of participants in a prodromal stage is unknown. Additionally, direct comparisons between groups of LRRK2-NM individuals and patients with PD are complicated by differences in age and sex, with patients with PD being older and predominantly male. These issues are common among studies of asymptomatic carriers of PD risk mutations, including *LRRK2* G2019S[29] and others[30–32] and are inherent due to the rarity and incomplete, age-related penetrance of such mutations. We chose to maximize statistical power and completeness of description by including all LRRK2-NM participants; prodromal neuropathology in eventual converters should augment oculomotor dysfunction, so our results may be attenuated by the presence of non-converters. We also chose to age- and sex-match our control cohort to the LRRK2-NM cohort as the comparison of LRRK2-NM to controls is more novel than to patients with PD; thus, differences between controls and patients with PD may partially relate to age and/or sex effects. However, although the PD group in the current study was significantly older and male-dominated, we replicated established behaviour of patients with PD[15].

Site-related variability in LRRK2-NM data is possible; however, most LRRK2-NM participants (58.8%) were recruited at Toronto Western Hospital (TWH), and all patients with PD were collected at TWH. We did not control for medication and cognitive impairment, which may modulate oculomotor behaviour[11,17–19]. We included LRRK2-NM participants and patients with PD with below-normal MoCA scores (<26) to maximize cohort size and capture the breadth of prodromal and manifest PD. Finally, although the presence of preclinical or prodromal PD pathology in the LRRK2-NM cohort may be the most obvious (and perhaps likely) explanation, it is possible that the mutation or other mutations inherited with it could act via a pathway other than PD-related neurodegeneration to produce the observed behavioural alterations. We consider cognitive impairment, medication, and other pathologies more thoroughly in the Supplementary Discussion.

In conclusion, oculomotor assessment is a promising technique to detect early behavioural abnormalities in prodromal PD, such as LRRK2-NM individuals. Here, we observed elevated short-latency prosaccades, reduced antisaccade pupil velocity, and less frequent prediction of fixation point appearance, which echoed the behaviour of patients with PD. Unexpectedly, we also observed elevated blink probability after correct prosaccades, opposite to patients with PD. Fixation acquisition behaviour is a particularly novel target of analysis whose neural correlates must be characterized. Its alteration in a prodromal cohort suggests its ability to detect mild pathology, especially cognitive in nature, and it is therefore an exciting focus of future study. The neural basis of increased rather than decreased blinking must also be illuminated.

These behavioural alterations represent avenues of future investigation to determine whether these metrics are abnormal in other PD prodromes and whether they predict PD conversion and/or track disease progression. Larger LRRK2-NM cohorts and longitudinal approaches will also be critical to future studies describing oculomotor aberrations in prodromal PD. At the individual level, these parameters could be assessed collectively (i.e. detecting a pattern of pathological behavioural shifts) to select candidates for disease-modifying clinical trials or to flag prodromal disease in clinic; their ability to accurately identify individuals in prodromal states will be an important future step. Applying large-scale machine learning approaches to construct automated diagnostic and tracking tools[59] may be an efficient method of patient-level assessment and therefore represents a favourable line of research. Furthermore, given the varying association between *LRRK2*-associated PD and α-synuclein-positive Lewy pathology, it will be essential to determine the relationship between oculomotor abnormalities and underlying pathological substrate, perhaps evaluated by α-synuclein seeding amplification assays[60].

Methods

Participants

This study was approved by the human research ethics boards of Queen's University Health Sciences and the University Health Network and is in accordance with the Canadian Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans and the Declaration of Helsinki. All participants provided informed written consent. Participant demographic and clinical characteristics are summarized in Table 1, Supplementary Fig. 1 and Supplementary Table 1. Participants reported current medication and completed the Montreal Cognitive Assessment (MoCA)[34] at time of testing, for which scores ≥26 are considered normal. Because the task paradigm required discrimination of green and red stimuli to indicate trial type, participants were required to have intact colour vision (no colour blindness). All participants are included in all analyses, except one patient with PD excluded from pupil analysis (see "Data analysis" below).

17 LRRK2-NM participants were identified through probands seen at the Edmond J. Safra Program in Parkinson's Disease and the Morton and Gloria Shulman Movement Disorders Clinic at Toronto Western Hospital (TWH). All participants underwent a standardized medical history and examination for motor and non-motor manifestations of Parkinson's disease (PD) by a movement disorders neurologist. Information regarding comorbidities was available for 13 of the 17 participants, all of whom reported no concurrent neurological or psychiatric conditions. LRRK2-NM participants completed the Beck Depression Inventory (BDI)[61], Geriatric Depression Scale (GDS)[62], and State-Trait Anxiety Inventory (STAI) (Form Y)[63]. Scores were available for 5 of 17 LRRK2-NM participants (except for STAI-Form Y-2, which was available for 6 LRRK2-NM participants). Scores generally indicated low levels of depression and anxiety; see Supplementary Table 1.

A prodromal score reflecting each LRRK2-NM participant's likelihood of prodromal PD was calculated according to published methodology[37] based on combined likelihood ratios (LRs) of multiple PD risk factors, including demographic (e.g. sex), environmental/lifestyle (e.g. pesticide exposure, smoking) and clinical markers (e.g. motor abnormalities, olfactory loss, autonomic dysfunction). Scoring higher than a cutoff equivalent to 80% probability of prodromal PD is considered "probable prodromal PD". This score can be used to determine individuals' probability of prodromal PD in both idiopathic and genetic cases since it incorporates established LRs for known PD risk mutations[37]; in LRRK2-NM individuals, it accurately predicts phenoconversion to manifest PD[64] However, note that some features (e.g. RBD, hyposmia)[7,8] are less frequent in patients with *LRRK2*-associated PD, which may slightly confound the interpretation of low scores.

Due to the rarity of *LRRK2* G2019S, data from LRRK2-NM participants was collected by transporting eye-tracking equipment to various North American locations near participants' homes to maximize accessibility: specifically, Toronto, ON; Winnipeg, MB; Middleton, WI; Halifax, NS; Chicago, IL; and West Palm Beach, FL.

Additionally, we included three LRRK2-NM participants with MoCA scores <26 to maximize statistical power and characterize the full spectrum of potential prodromal PD.

8 patients with PD were recruited specifically for this study through the Edmond J. Safra Program in Parkinson's Disease and the Morton and Gloria Shulman Movement Disorders Clinic at TWH. A further 39 patients with PD were recruited at TWH for the Ontario Neurodegenerative Disease Research Initiative (ONDRI) study[65]. All were diagnosed by a movement disorders neurologist according to UK Brain Bank criteria[1] and were confirmed non-carriers of G2019S. Patients with PD remained on their regular dopaminergic medication throughout the experimental protocol. Two patients who otherwise would have met inclusion criteria were taking cholinergic medications known to affect pupil responses and were therefore excluded from the study (not reflected in participant count). For comparability with the LRRK2-NM cohort, we did not require MoCA scores ≥26; 13 patients with PD scored below this cutoff. Patients with PD also completed the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III, which assesses motor function; scores are reported in Table 1 and indicate generally mild severity. Some scores assessing psychiatric symptoms were also available in patients with PD, although the specific inventories completed varied between patients. The 8 patients with PD recruited specifically for this study at TWH completed the BDI[61], GDS[62], and STAI-Form Y[63]. Scores were available for 7 of 8 TWH patients with PD (except for STAI-Form Y-2, which was available for 6 patients with PD). The remaining 39 patients with PD, who were recruited at TWH as part of the Ontario Neurodegenerative Disease Research Initiative (ONDRI), completed the Quick Inventory of Depressive Symptomatology (QIDS)[66] and Generalized Anxiety Disorder 7-item (GAD-7)[67]. Scores were available for all 39 ONDRI participants. As in the LRRK2-NM cohort, scores indicated low levels of depression and anxiety; see Supplementary Table 1.

Control participants were recruited from the community of Kingston, ON, Canada as part of a larger control cohort study[14,35] and had no known neurological or psychiatric conditions. We included the next two youngest and next two oldest control participants from this cohort, with MoCA scores ≥26, who were of the same sex as each LRRK2-NM participant. This resulted in four matches per LRRK2-NM participant; we then eliminated duplicate matches for a final cohort size of 63 controls (Supplementary Fig. 1). Due to inherent differences in age and sex between LRRK2-NM and PD participants (PD older and predominantly male) that prevented accurate matching of a single control group to both cohorts, we opted to match only to the LRRK2-NM cohort given the novelty of this comparison and the need to accurately describe behavioural alterations in LRRK2-NM participants relative to similar controls without the mutation. Additionally, we required MoCA scores ≥26 to minimize controls' likelihood of prodromal neurodegenerative disease.

Genotyping

Blood samples were drawn from all LRRK2-NM participants and patients with PD, and blood extraction kits (Qiagen, Venlo, Netherlands) were used to isolate DNA. The absence of *LRRK2* G2019S mutations was confirmed in ONDRI participants with PD using a custom-designed next-generation-sequencing-based panel (ONDRISeq)[68]. For all other patients with PD and all LRRK2-NM participants, mutation absence or presence respectively was confirmed using polymerase chain reaction (PCR) amplification and restriction enzyme digest with SfcI followed by gel electrophoresis and subsequently validated using Sanger sequencing.

Eye tracking: recording and apparatus

Gaze position, pupil size, and eye blinks were detected monocularly using an infra-red video-based eye-tracker (EyeLink 1000 Plus, SR Research Ltd., Ottawa, ON, Canada) at a sampling rate of 500 Hz. Participants sat in a dark room, with their heads resting comfortably in a chin and forehead rest, 60 cm away from a 17-inch LCD monitor with 1280 × 1024 pixel resolution and 60 Hz refresh rate. A nine-point grid array calibration and validation procedure (maximum 30 min duration, although it was much shorter for most participants) was performed prior to task initiation, with use of a five-point grid if nine-point was too difficult; validation error <1° was required for task initiation. To minimize spurious luminance-related effects on pupil responses, a spectrometer was used to confirm equal monitor luminance across apparatuses and all testing was conducted in windowless rooms with internal lighting off. Representative saccade traces from three controls, three LRRK2-NM participants, and three patients with PD are displayed in Supplementary Fig. 2.

Task paradigm

Participants completed an interleaved pro- and antisaccade task (IPAST)[69] consisting of two blocks of 120 trials approximately 7 min each in duration. In brief, prosaccade and antisaccade trials are pseudo-randomly interleaved; both trial types begin with a fixation period during which a central fixation point (FP) is displayed. The FP colour indicated the trial type (1000 ms duration, 0.5° diameter; 44 cd/m^2; green: prosaccade; red: antisaccade). The fixation period was followed by a 200 ms gap period displaying a blank screen, then a peripheral stimulus (1000 ms duration, 0.5° diameter; 62 cd/m^2) appearing 10° left or right of centre. Participants were instructed to look at the peripheral stimulus on prosaccade trials but to look in the opposite direction on antisaccade trials. Left and right stimulus positions were also pseudo-randomly interleaved. Following the presentation of the stimulus, it disappeared and was followed by a 1000 ms period between trials (intertrial interval; ITI) during which the screen was blank. The next trial would then begin with the reappearance of the FP.

Data analysis

Trials were categorized by in-house auto-marking code (MATLAB, MathWorks Inc., Natick, MA, USA) according to the direction and reaction time of the first saccade[69]. Trials with excessive tracking loss, saccades to random locations, very delayed saccades (reaction time >800 ms), or failure to fixate (participant never fixated within a circular window of radius 2.5° around the central FP while it was displayed onscreen) were excluded from analysis of saccade behaviour. Note that trials in which the participant fixated the central FP, broke fixation, and then returned were permitted. Of a total 29,874 trials completed across all groups, 1355 (4.5%) were excluded from analysis due to these reasons. The mean percentage trial loss per person was 3.2% in the control group (SD: 5.9%), 2.3% for LRRK2-NM participants (SD: 3.8%), and 7.3% for patients with PD (SD: 9.0%). The remaining trials were categorized into correct responses (towards stimulus for prosaccades, away for antisaccades) or direction errors (away from stimulus for prosaccades, towards for antisaccades); curves displaying the proportion of each response type as a function of saccadic reaction time (SRT) were constructed and used for further analysis (Fig. 1). Due to the 90 ms required for visual signals to propagate through circuitry and generate a saccade, any responses initiated from −110 ms to 89 ms relative to stimulus appearance would occur when the participant perceived the screen to be blank and represent guessing behaviour, termed "anticipatory saccades"[69]. Express-latency saccades, the fastest possible visually-driven saccades, were defined from SRT 90–139 ms[56,70]; any saccades with SRT 140–800 ms were considered regular-latency. Viable-latency saccades encompassed both express- and regular-latency saccades.

Raw pupil area in pixels was measured continuously over the fixation and gap periods when the eyes were stationary[69]. FP appearance induced a standard pupil response: constriction followed by dilation prior to stimulus appearance[13,69]. Continuous curves showing pupil size over time during the fixation period for correct prosaccade and

antisaccade trials were created; these were also used to compute pupil velocity curves. We removed the effect of inter-individual variation by normalizing the curve for each trial to its baseline pupil diameter (measured as the mean pupil diameter from 150 to 200 ms after FP appearance). We also analyzed only the dilation component of the pupil response by instead normalizing to maximum constriction (i.e. nadir of standard pupil response). One patient with PD was excluded from pupil analyses because they had no correct antisaccade trials free of blinking or saccades during the pupil measurement epoch.

Blinks during video-based eye tracking occlude the pupil, producing tracking loss with large fluctuations in pupil area before and after. An automated blink detection algorithm implemented in MATLAB[69] was used to identify this pattern to distinguish blinks from other data loss during the ITI, fixation, gap, and stimulus periods. Blink probability was computed continuously over time, like previous studies[14,71] that incorporate changes in both blink rate and duration. We performed this analysis for all trials pooled as well as correct prosaccades and antisaccades separately.

After the peripheral stimulus disappears, participants must return their gaze to the centre of the screen to view the FP providing the task instruction for the next trial. We termed this returning of gaze to the centre "fixation acquisition" because participants must acquire fixation on the task-relevant central location of the FP, and defined it as the first time the participant's gaze fixated on the future location of the FP or on the FP itself. We extracted the latency of this behaviour relative to stimulus appearance (e.g. a fixation acquisition latency of −1800 ms indicated that the participant first returned gaze to the screen centre 1800 ms before the peripheral stimulus appeared, which fell during the ITI and therefore represented a predictive acquisition of fixation occurring before FP appearance). To generate continuous curves representing latency of fixation acquisition, we computed cumulative curves like those produced for saccades. We performed this analysis for all trials pooled as well as correct prosaccades and antisaccades separately. Note that because "fixation acquisition" refers simply to the latency at which participants returned gaze to the centre of the screen, it is unrelated to the stability of the subsequent fixation itself and is therefore not a measure of or affected by behaviours such as square-wave jerks or other involuntary saccadic intrusions.

Statistical analysis

Most oculomotor behaviours assessed in this study were processed into continuous, time-series data, as described above. For these data, we computed a two-tailed Wilcoxon rank-sum test at each sampled time point between each pair of groups (LRRK2-NM participants vs controls, patients with PD vs controls, LRRK2-NM participants vs patients with PD), which produced clusters of adjacent significant time points whose test statistics could be summed to produce a single statistic for each cluster. To correct for multiple comparisons and the autocorrelative nature of time-series data, we performed a cluster-based permutation analysis that was originally developed for EEG data[72] but has since been applied to eye tracking analyses[73,74]. Briefly, the group labels are shuffled and Wilcoxon analysis reapplied 1000 times. For each iteration, we saved the maximum cluster sum and used it to construct a distribution of sum statistics under the null hypothesis; we then considered clusters with sum statistics outside the 2.5%–97.5% range of the distribution to be significant (i.e. $p \leq 0.025$). We show the results of this analysis via horizontal tracks on figures displaying their respective data, with dark filled bars indicating significantly different periods ($p \leq 0.025$) between pairs of groups. We have also chosen to show 'trending' periods ($p < 0.05$) using lighter filled bars; we consider this important due to the reduced power of cluster-based permutation analysis to detect smaller clusters as the null distribution is constructed of only the maximum sum statistic from each permutation[74]. Smaller clusters might imply subtler or shorter-duration effects that, although not strong enough to reach statistical significance in the current study, could still be meaningful for future holistic characterization of oculomotor behaviour in LRRK2-NM individuals. Wilcoxon testing and cluster-based permutation testing were performed in MATLAB (MathWorks Inc., Natick, MA, USA).

We evaluated between-group differences in age using a one-way ANOVA with post hoc Tukey's HSD test. Because they were non-normally distributed (assessed using visual inspection and Shapiro-Wilk test for normality), MoCA scores were compared using a Kruskal-Wallis test with post hoc Dunn-Bonferroni test. Between-group differences in sex were evaluated using a chi-squared test. Effect sizes are reported as η^2 for ANOVA and Kruskal-Wallis tests ($((H\text{-}k+1)/(n\text{-}k)$ for Kruskal-Wallis, where H = test statistic, k = number of groups, and n = total observations), and Cramer's V for chi-square tests ($V = \sqrt{(\chi^2/(n^*df))}$, where χ^2 = test statistic, n = total observations, df = degrees of freedom). All ANOVA, Kruskal-Wallis, and chi-square tests were performed in SPSS version 29 (IBM, Armonk, NY, USA).

'Traditional' oculomotor analysis

Finally, as a point of direct comparison to previous studies, we report between-group analyses of commonly published saccade parameters, such as amplitude, reaction time, and error rates. Most of these results are found in Supplementary Tables 2–4, although we report saccade amplitude in the main text below given its known alteration in patients with PD and our inability to assess it using continuous time-series data.

For saccade behaviour, we computed percentages of anticipatory saccades, express-latency correct prosaccades and antisaccade errors, and regular-latency antisaccade errors, as well as mean reaction times of viable correct pro- and antisaccades. Fixation breaks were considered to occur when a saccade was made away from the central fixation point, without returning to it before its disappearance; we also computed percentages of these. Finally, we computed the mean amplitude and peak velocity of viable correct prosaccades. Voluntary override time (VOT) was determined as previously described[11] and indicates the time at which voluntary processes generating correct antisaccades begin to outcompete automated ones driving errors. Saccade results are displayed in Supplementary Table 2.

Pupil metrics were calculated as described previously[69]. These were computed for correct prosaccade and correct antisaccade trials separately. Pupil baseline was measured by taking the mean pupil size during a window 150–200 ms after fixation point appearance. Pupil response latency refers to the time between fixation point appearance and the beginning of the constriction response. The constriction phase of the pupil response therefore lasts from the onset of pupil response to the time of maximum constriction. Pupil constriction time refers to the time taken to reach maximum constriction from fixation point appearance; pupil constriction size is the change in pupil size between baseline and maximum constriction. Peak constriction velocity is the maximum velocity reached during the constriction phase. The pupil dilation phase lasts from the time of maximum constriction to the appearance of the peripheral stimulus; pupil dilation size refers to the change in pupil size during the dilation phase, and peak pupil dilation velocity refers to the maximum velocity reached during the dilation phase. Results are displayed in Supplementary Table 3.

We computed overall blink rate by dividing the total number of blinks by total minutes spent performing IPAST. We also computed blink rates during the intertrial interval (ITI, the 1000 ms period between stimulus disappearance and fixation point appearance for the subsequent trial) and fixation period (1000 ms duration). Blink results are displayed in Supplementary Table 4.

Differences between groups were assessed using one-way ANOVA with post hoc Tukey's HSD test for normally distributed variables and Kruskal-Wallis test with post hoc Dunn-Bonferroni test for non-normally distributed variables (assessed via visual inspection and Shapiro-Wilk test). Additionally, in the LRRK2-NM cohort, we computed Spearman correlations between prodromal scores and "traditional" oculomotor parameters (Supplementary Table 5).

Data availability

Data pertaining to participants recruited via ONDRI is available from the Ontario Brain Institute (OBI) upon request. Data pertaining to controls, *LRRK2* mutation carriers, and patients with PD recruited specifically for this study is available from the authors upon request.

Code availability

Code used to analyze data described in this manuscript is available from the authors upon request.

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Author contributions

A.E.L., C.M. and D.P.M. conceptualized and designed the study. D.G.D., L.A.G., N.A., J.E.P., Ja.H. and T.G. acquired the data. B.C.C., Je.H. and D.C.B. completed data preprocessing. H.C.R., I.C.P., D.C.B. and B.C.C. developed statistical analysis. H.C.R. performed analysis. H.C.R., N.P.V., C.M. and D.P.M. interpreted the data. H.C.R. and J.E.P. wrote the initial draft of the manuscript. H.C.R., N.P.V., D.G.D., A.E.L., C.M. and D.P.M. revised the manuscript.

Competing interests

D.C.B., B.C.C. and D.P.M. are co-founders of Dynamiris Inc., which is currently developing a clinical tool for neurological and psychiatric disease diagnosis using eye movement data such as that described in this study. All other authors have no other competing interests.

Additional information

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